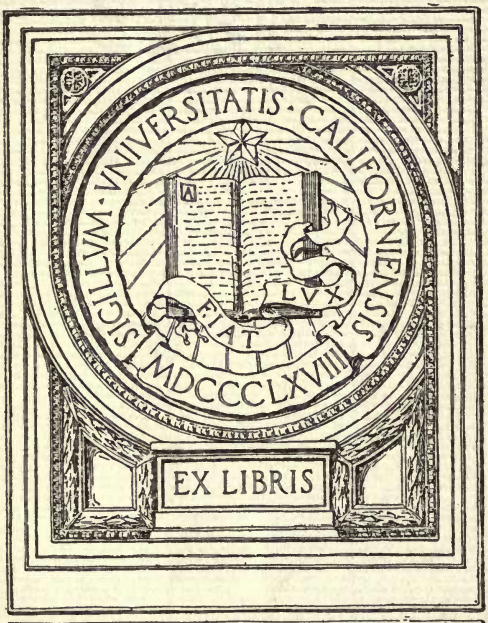

THE RESULTS OF SELECTION WITHIN PURE LINES OF *PESTALOZZIA GUEPINI* DESM.

By

CARL DOWNEY LA RUE

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THE RESULTS OF SELECTION WITHIN PURE LINES OF *PESTALOZZIA GUEPINI* DESM.[1]

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Received June 30, 1921

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INTRODUCTION

Since JOHANNSEN's classic experiments on beans led him to formulate his theory of the multilinear composition of species and the inefficacy of selection within any of the lines, several workers have studied the effect of selection within pure lines of various other organisms. No apology need be offered, however, for this extension of the work to one of the asexually reproducing Fungi Imperfecti, for the peculiarities of the material make possible a quite different experimental attack. Inasmuch as the literature of the selection problem has recently been thoroughly reviewed by JENNINGS (1916, 1920) and others, only those papers will be considered here which have special bearing on the present investigation.

One of the most noteworthy of such contributions is that of JENNINGS (1908) on Paramecium. In a carefully planned and executed series of experiments in which numerous selections were made, JENNINGS was unable

[1] Papers from the Department of Botany of the UNIVERSITY OF MICHIGAN, No. 186. A portion of this investigation was carried out at Kisaran, Asahan, Sumatra; the remainder in the Botanical Laboratory of the UNIVERSITY OF MICHIGAN.

to detect any effect of selection. This was among the first of the considerable series of investigations which appeared to bear out the JOHANNSEN theory.

The work of EWING (1914 a, 1914 b, 1916) is of particular interest because of the large number of generations during which he practiced selection. He studied an aphid, *Aphis avenae*, and found that selection, even for eighty-seven generations, was totally without effect.

The work of HANEL (1908) has received much attention and adverse comment. HANEL concluded that selection was without effect on Hydra, but it is doubtful whether the experimental results justified the conclusion. More recently the status of HANEL'S work has been discussed and the experiments have been repeated by LASHLEY (1915, 1916). The latter worker took account of the sources of error in HANEL'S experiments and carried out so excellent an investigation as to leave no doubt that selection within the genus Hydra is ineffective.

A number of other investigators have secured results which substantiate the work of JOHANNSEN. Some of them have made careful investigations which are of considerable value. In other cases the results of loosely planned and brief experiments have been recorded. For one to believe, because selection for one generation within a line has had no effect, that selection is therefore in all cases ineffective, requires a very sanguine temperament. There was ground for the protests of PEARSON (1910) and HARRIS (1911) that the pure-line theory had been too readily accepted before adequate evidence had been presented. However, all in all, a considerable body of data supporting JOHANNSEN has been secured, some of it exceedingly hard to refute. Until recently the results of all investigations seemed only to add more evidence in support of the theory.

Within the last few years, however, more and more conflicting evidence has been amassed, partly through critical analysis of the older data, and partly by new experimentation. JOHANNSEN'S experiments have been criticized because they were not carried on for a sufficiently large number of generations. By statistical analysis PEARSON (1910) found that there was some evidence that JOHANNSEN'S results really showed inheritance of somatic variations within pure lines. ROOT (1918) has discounted EWING'S results because the organism concerned is one which in nature is highly stable, its lack of variability affording at the start little ground for hope that new strains could be produced.

Several objections have been offered to the conclusions of JENNINGS from his work on Paramecium. One is that he studied size characters which are highly variable under different environmental conditions. These

characters also vary with different stages of maturity of the individuals and it is admitted by JENNINGS himself that one cannot be certain that only mature specimens are being measured.

Turning now from reinterpretation of old work to the newer experimental data, we find that several studies have been interpreted as showing positive selection effects. Thus STOCKING (1915) found that it was possible to produce distinct groups by selection within certain lines of abnormal Paramecia. MIDDLETON (1915) was able to produce strains of Stylonychia, distinct in regard to rate of fission, and this almost at will. JENNINGS (1916), with much greater difficulty, detected small changes in Difflugia, which he attributed to selection. ROOT (1918) and HEGNER (1919), working with Centropyxis and Arcella, respectively, secured data in conformity with those of JENNINGS with Difflugia.

To this later work, however, certain objections have been made. STOCKING'S results were obtained with abnormal forms and it seems to have been generally concluded that normal variations might not necessarily behave in the same way. MAST (1917) secured two groups distinct for rate of fission in *Didynium nasutum*, without selection, presumably by a mutation in one group. This has some bearing on MIDDLETON'S results, and if it be argued that very frequent mutations would be needed to account for the rapid changes produced by MIDDLETON'S selections, it may be pointed out that great instability must obtain in an organism to allow of such changes even by selection.

MORGAN (1916) suggests that the selection results with Difflugia may be due to random distribution of discrete particles of nuclear material (chromidia) to the pairs of offspring. If such were the case, and if the bearers of particular hereditary characters were contained in the chromidia, one would not expect the effects of selection to be indefinitely cumulative. Moreover, the variations would necessarily be of a discontinuous type, and perhaps remotely comparable to non-disjunctional mutations or somatic mutations. The same suggestion would also apply to the results of selection in Centropyxis and Arcella. In all three cases it is likely that the variations dealt with are not of the usual continuous type.

LASHLEY (1915, 1916) has produced evidence that PEARSON'S criticism of JOHANNSEN'S work is not well founded, and that the parent-offspring correlation is not reliable as a criterion in selection work.

It must be obvious that the question as to whether variations occurring within a pure line are purely somatic and non-heritable or not, is one that is far from a definite answer. Further investigations on forms suitable for the study of the problem, wherever they may occur, are greatly needed.

Thus far Protozoa have been used in most of the critical and extensive investigations, and these organisms are not only subject to great environmental effects, but give rise to only two offspring at a time,—a serious disqualification for the most accurate results. JOHANNSEN'S original material, the garden bean, was ideal from the standpoint of number of offspring, but, reproducing sexually, it offered a possibility of Mendelian segregation obscuring the true fluctuation which it was desired to study. The Fungi Imperfecti seemed to afford an ideal material, combining the advantages of numerous progeny with uniparental, non-sexual reproduction.

REASON FOR THE CHOICE OF PESTALOZZIA

Pestalozzia Guepini Desm., one of the Fungi Imperfecti, was chosen by the writer as a species unusually well suited for use in the study of the selection problem. The species is polymorphic, containing a number of different strains, so that it is not unlikely that new strains are being formed at the present time. It is very easily grown in culture in the laboratory and does not require tedious, special methods. Sporulation occurs readily in culture, and development from the spore to the fruiting stage requires only a few days, so that generations can be grown in rapid succession. Spores are produced in enormous numbers so that more than enough are always available for observation. They possess two characters that are capable of accurate measurement, and a change in color at maturity enables one to tell with certainty which spores are mature and which immature. Size characters may therefore be studied without fear that forms apparently small are only immature. In addition to these advantages the spores are produced, as in all Fungi Imperfecti, entirely asexually, so that there is no possibility that the results are influenced by sexual phenomena.

Pestalozzia Guepini Desm. is widely distributed in the eastern tropics, where it causes the gray-blight disease of tea, and leaf-spot diseases of cocoanut, betel nut palm, African oil palm, Para rubber, and doubtless many other wild and cultivated plants of those regions. The species contains a number of forms which differ more or less markedly from one another in morphological characters. Some of them are likewise supposed to be restricted to particular hosts. Strains occurring in regions far removed from areas known to contain *Pestalozzia Guepini*, and on hosts not previously reported for this fungus, have been described as new species. This is only natural, for the species was originally not closely defined. The new species however, are even less perfectly described than *P. Guepini*, so that one gains nothing by using their names. For the purposes of this

study the concept of *P. Guepini* employed is that given in a preliminary study of the strains of the species by LaRue and Bartlett (1922).

In this investigation the authors found that from thirty-five isolations of Pestalozzia from the Para rubber tree, the cocoanut palm, the African oil palm, the betel palm, and the tea plant, at least fourteen strains, distinct in regard to morphological characters, could be distinguished. More minute morphological studies would in all probability have resulted in the division of some of these strains into smaller distinct groups. Cross inoculations might possibly have revealed variations in regard to hosts infected, and physiological studies would almost certainly have shown that some isolations differ physiologically from others in the same morphological group. The large number of forms found within the comparatively limited territory on the East Coast of Sumatra, from which the cultures were secured suggests that an equally large number of strains might be found in other regions, many of them doubtless distinguishable from those already studied. The formation of at least one new strain by mutation during the period covered by the present investigation of the effect of selection leads to the belief that other strains are now being formed in nature and that *P. Guepini* is a species in what may be called a plastic condition. In this respect it is comparable to the organisms studied by other recent workers who have sought to find a clue to the process by which evolution proceeds.

Only the spores of the fungus were studied minutely by LaRue and Bartlett (1922) in the investigation of variability in *P. Guepini* mentioned above. The vegetative characters and growth habits also show considerable variation but these are much more limited in range and more difficult to measure than the quantitative characters of the spores, and therefore were not utilized. For selection studies also, the spore characters were chosen as most suitable though other characters might have given equally satisfactory results had they been more easily measured.

The spindle-shaped spores of *Pestalozzia Guepini* are composed of five cells of which the three central ones are smoky or black at maturity, while the other two are hyaline. The distal hyaline conical cell normally bears at its tip three slender, unjointed, hyaline appendages which diverge so that their tips are widely separated. Spores are occasionally found which contain three or four cells, and others which bear two appendages or even only one, but these are apparently aberrant forms. Thus far no strain has been found which is constant for any of these peculiarities, and in the cases where the aberrant spores have been tested, they have produced normal progeny.

The total range of mean length of spores for all the strains of *P. Guepini* isolated by LaRue and Bartlett (1922) was from 19.9μ to 28.3μ. Figure 1 shows the polygons of variation in spore length for a few representative strains. Mean appendage lengths of the strains ranged from 10.9μ to 30.0μ. The polygons for variation in appendage length are shown for six representative isolations in figure 2. More complete data, which cannot be reproduced here, are given in the paper already cited. In view of the large number of strains which exist within the species, this fungus is a very favorable organism for use in making an attempt to develop still other strains by the selection of variant spores.

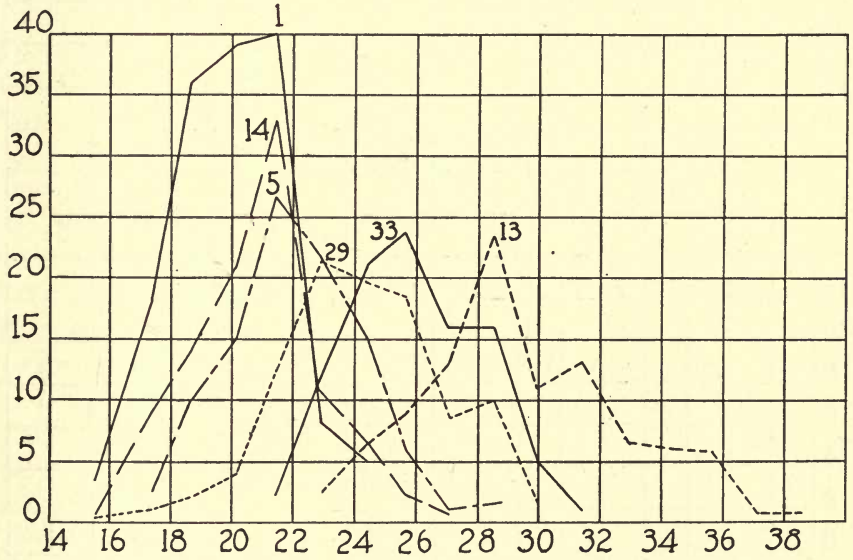

Figure 1.—Polygons of variation in spore length for six strains of *Pestalozzia Guepini*. The ordinates are percentages; the abscissae, lengths in μ.

It is essential that any organism which is to be employed in the study of the selection problem be easy to cultivate and in this respect *P. Guepini* is admirable. It will grow readily and rapidly on almost any common nutrient agar and can also be grown on sterilized leaves, twigs and fruits of its common hosts. In Sumatra usually only about four days time was needed to develop the fungus from spore to the fruiting condition. In Michigan a slightly longer time was needed to secure spores even when the fungus was kept at a temperature approximately the same as that of Sumatra. Strong light appears to inhibit the growth of the young mycelium, but exposure to light after the mycelium is about three days old seems to hasten the production of spores.

When sporulation begins the spores are developed very rapidly and in enormous numbers. This is a characteristic exceedingly valuable in biometrical studies, since the variates are produced under as nearly identical conditions as may be found anywhere. By measuring a sufficient number of such spores one can get the whole range of variability induced by the reaction of a given set of environmental conditions with the hereditary characters of the organism. In an organism which produces offspring slowly a number of fluctuations in environment must necessarily take place

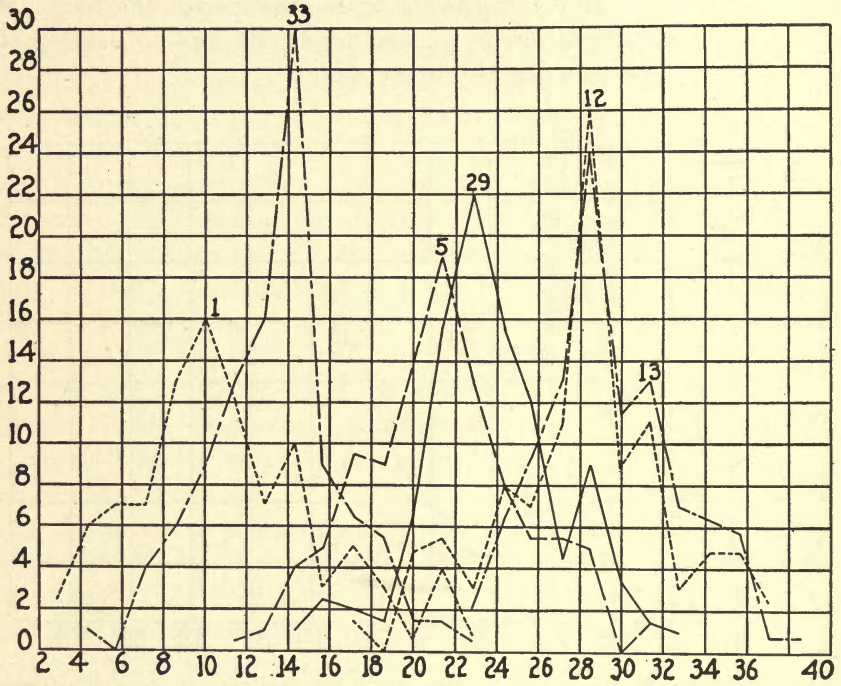

FIGURE 2.—Polygons of variation in length of spore appendages for six strains of *Pestalozzia Guepini*. The ordinates are percentages; the abscissae, lengths of appendages in μ.

before a number of individuals sufficient to give a reliable mean is produced. This complicates the situation greatly and the means of two groups of organisms so produced are less readily comparable than those developed in an organism such as Pestalozzia.

Mention has already been made of the dark color of the three central cells of mature spores of *P. Guepini*. This color is a valuable index of the age of the spores because it does not fully develop until the spores have reached their full size and the appendages have been fully formed. One can always recognize mature spores at a glance and is thus able to reject

all immature stages of development. This completely obviates one great difficulty usually found in the study of size characters, that of determining which individuals are really small, which only immature. This difficulty seems insurmountable in such forms as Paramecium, but in Pestalozzia, as in Difflugia, the nature of the organism allows size characters to be observed without fear of complications from this source.

A further word of explanation may be desirable with regard to the assumed absence of sexual reproduction in Pestalozzia. As has been stated, it belongs to the group of Fungi Imperfecti. Many of the species in this miscellaneous assemblage have been found to have other stages in their life-history which have enabled them to be classified under other groups. With few exceptions these other stages of Fungi Imperfecti have shown them to be degenerate members of the Ascomycetes. Many Ascomycetes have both the ascus stage, which follows a sexual fusion, and an asexual, conidial stage. It is commonly assumed that the conidial forms, which make up the Fungi Imperfecti, either have, or have had at some time, a stage with a higher spore form. Whether Pestalozzia now has an unknown ascus stage is not so important, as is the probability that if it had a sexual stage at all, it would be indicated by the appearance of a readily recognizable ascus stage. No such form has ever been observed in the hundreds of cultures grown in this series of experiments and it is reasonably certain that it never appeared. From the data gained from the study of analogous forms we can safely assume, (1) that the conidia of Pestalozzia are entirely asexual, (2) that if a sexual stage appears it will result in the production of an ascus form and therefore will be easily detected, and (3) that we have no need to fear that obscure nuclear recombinations are complicating the results secured.

The unique combination of characters possessed by Pestalozzia and discussed above renders the organism an unusually suitable one for use in study of the results of selection within vegetative lines. Because the fungus was so well adapted for this use and because no similar material had ever been used for this purpose the author was led to undertake the investigation here described. Presented in review, the characters which make Pestalozzia specially desirable for such use are: (1) The presence of numerous distinct strains within the species; (2) the ease with which it is grown in culture; (3) the rapidity with which consecutive generations may be produced; (4) the availability of at least two easily measurable independent characters; (5) the rapidity with which spores are produced and the enormous numbers of spores produced, which enable one to secure significant statistical constants for each generation; (6) the dark colora-

tion of the three central cells of the spore which appears only at maturity, and serves as a criterion for the elimination of mere growth stages; (7) the total absence of any sexual form of reproduction.

EXPERIMENTS

Methods and conditions

Since the aim of the investigation was to study variations of genetical significance, every reasonable attempt was made either to remove variations in environment or to provide controls for them. All the cultures used were grown on agar in tubes, and under ordinary laboratory conditions. Aside from the fact that it would be extremely inconvenient, if not impossible, to control a selection experiment in which the organism was grown as a parasite on its usual host, such a procedure would doubtless offer more variations as to food supply, etc., than would growth on culture media. WOLLENWEBER (1914) has shown that organisms which are facultative parasites show as normal development in culture as on their usual hosts.

That fungi, especially Saprolegnias, may be exceedingly sensitive to variation in food supply has been shown by KAUFFMAN (1908) and PIETERS (1915). However it is not believed that Pestalozzia is so exquisitely sensitive to such variations as are some other fungi. In any event the variations thus introduced into this work are doubtless much smaller than those which of necessity apply to any other organism which has been used in the study of selection up to the present time.

The nutrient solution was all made up before the experiment was begun. Tender twig tips and young leaves of *Hevea brasiliensis* were boiled in water and a quantity of brown sugar made from the sugar palm (*Arenga saccharifera*) was added. The decoction was strained, concentrated by boiling, and after thorough mixing to secure uniformity, was poured into flasks which were plugged with cotton stoppers and sterilized.

The single lot of medium thus prepared was stored and used throughout the experiments until the writer's return to the United States. (A supply of the medium was brought to America but was lost in transit, so that it was necessary to supply a substitute, as will be noted later.) For growing the cultures nutrient agar was made up according to the following formula:

Hevea decoction,	250 cc
Water,	3750 cc
Agar-agar,	120 gm

The medium was very thoroughly mixed before being put into tubes, and the same amount was placed in each tube. In making cultures care was taken that all the tubes of a given generation were poured from agar of the same lot.

The cultures were not kept in a constant-temperature chamber but the range of variation in temperature and humidity is not great in the east coast region of Sumatra. For the greater part of the year the daily change in temperature is from about 23° C to about 32° C. The total range of fluctuation for one five-month period of the selection experiment was from 19.4° to 33.9° C. During the same period the moisture content of the air varied from 52 percent to 100 percent with a usual daily range of approximately from 60 percent to 100 percent. In general the climate of Asahan varies so little from day to day, or from month to month, that it would be hard to find any place more uniform. This uniformity allows cultures to be carried on indefinitely without danger of extremes of heat or cold such as have ended various experiments of other investigators.

The selection experiments were made according to two plans. In the major part of the work the line of descent was determined wholly by divergence in progeny means without regard to the visible characters of the spores chosen as generation parents. In other words, the direction of selection in the chief experiments was guided by the *performance* of particular spores, rather than by their *appearance*, but the method was checked by parallel experiments in which the usual basis of selection, namely, selection of visibly divergent individual spores, was employed.

The characters concerned were length of spore and length of spore appendage. That these characters are heritable to a very considerable degree is shown by the fact that strains, distinct for either spore length or for length of spore appendages, have been isolated, and that these strains remain distinct from generation to generation in spite of fluctuations in environmental conditions. The heritable differences between the different strains are very considerable as may be seen from figures 1 and 2, and these differences make them especially promising for use in a study of the results of selection.

The number of cells in the spore and the number of appendages per spore might appear to be characters suited for study but this was soon found not to be the case. The number of cells in the spore shows little variation; the smallest number found in observing hundreds of thousands of spores was three, and the largest number seen was nine. In two cases where spores having one more than the normal number of cells were

selected it was found that the spores produced by the mycelia from these spores were entirely normal in regard to cell number.

The range of variation in number of spore appendages is even less than that in cells. The greatest number recorded from all observations made was four and the smallest number, one. Three different spores were isolated which bore only one appendage each. However, when these spores germinated and the mycelia from them produced spores, the latter were found to have the normal three appendages. Another spore bearing two appendages was isolated with the same result, and still another bearing four appendages gave progeny which showed no sign of this abnormality. Apparently variation in cell number and spore appendage number are not greatly different in behavior from sundry other abnormalities which appear from time to time in Pestalozzia, none of which appears to be hereditary.

Selection of spores according to their progeny

The method of procedure in selecting spores according to their progeny was as follows: A single spore culture was made from a given strain. When this culture produced mature spores agar plates were poured with various dilutions of these spores. A mass of spores was taken from the culture with a platinum wire and mixed with about 10 cc of sterile water in a sterile tube. From this water suspension of spores two loops were put into a tube of melted agar and thoroughly mixed. Two loops of this agar were then mixed in the next tube; and again two loops were used to inoculate the third tube. The agar from each tube was then poured into a sterile petri dish, and the dish was covered with a bell jar. When the spores germinated the agar plates were examined and spores were cut out from the most suitable ones and put into separate tubes of Hevea agar. Microscopic examination was made in each case to make sure that only one spore was used in inoculating each tube. On this account it was found more convenient to use a 1-percent beef-extract agar instead of Hevea agar for pouring the plates. It was not always possible throughout the experiments to regulate the number of spores in the poured plates. The third dilution plate was the one from which the spores were usually taken, but whenever this contained too few spores to make a full set of cultures for a given generation, the second dilution plate was used instead.

All the tubes contained agar made in one lot, tubed and sterilized at the same time, and stored under the same conditions. The transfers of the twenty single spores were made one immediately after the other, so that the time of transfer of each was so nearly the same as to admit of no reasonable assumption that this variation could be of effect. The twenty

tubes were put into one rack so that all might be subject to the same general environmental conditions.

When the mycelia had all grown and the spores were mature, 100 spores of each culture were measured and the mean spore length, or mean appendage length, as the case might be, was computed. By trial it was found that 100 measurements were sufficient to secure a smooth distribution

TABLE 1

Typical frequency distribution of measurements of spores and spore appendages from different cultures of Pestalozzia Guepini. Measurements in μ.

SPORE LENGTHS				SPORE APPENDAGE LENGTHS			
Length	Experiment 1 Plus group Generation 3 Culture 3	Experiment 1 Plus group Generation 3 Culture 8	Experiment 1 Minus group Generation 4 Culture 3	Length	Experiment 1 Minus group Generation 6 Culture 1	Experiment 2 Plus group Generation 1 Culture 1	Experiment 2 Plus group Gen. 1 Cul. 15
19.8	1	2	3	10.8	2		
20.7	1	2	3	12.6	2		2
21.6	4	5	8	14.4	3	3	1
22.5	8	9	11	16.2	8	5	7
23.4	11	17	15	18.0	15	11	12
24.3	12	19	17	19.8	17	12	15
25.2	19	14	15	21.6	16	15	18
26.1	14	12	12	23.4	13	17	14
27.0	8	9	6	25.2	10	14	10
27.9	8	4	3	27.0	7	14	8
28.8	8	4	2	28.8	5	5	6
29.7	2	1	2	30.6	2	3	3
30.6	2	2	2	32.4		1	3
31.5	1		1	34.2			1
Means	25.54±.15	24.79±.15	24.53±.16		21.22±.21	22.81±.27	22.42 ±.31
Standard deviations	2.18	2.18	2.38		4.61	3.96	4.57

curve and a mean with a satisfactorily small probable error, so with some exceptions, where a few more spores were taken, one hundred from each culture were measured. Typical distributions are shown in table 1.

After the twenty cultures were measured the one with the greatest mean spore length was chosen as the starting point for plus selection. Similarly the culture with the smallest mean spore length was taken as the beginning

of the minus selection. Another culture, which had a mean spore length as nearly midway between the other two as could be found, was chosen as the origin of an intermediate line to be carried on from generation to generation without selection.

From each of the chosen cultures plates were now poured. When the spores had germinated, ten single spores were cut out from the plates made from the plus-selection culture and the same number from the minus-selection culture. From the intermediate culture only one single-spore culture was made. See figure 3.

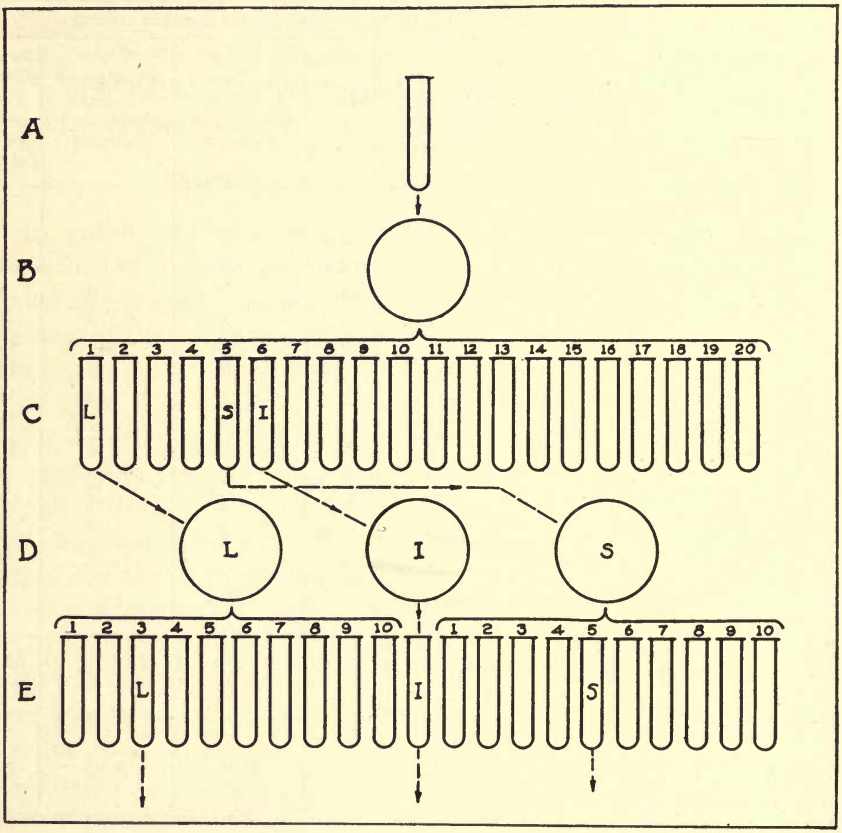

FIGURE 3.—Plan of method of selection used in experiments 1 and 2. A represents the parent culture; B, the agar plate poured with a dilution of spores from A; C, the daughter cultures grown from spores from B, of which L has the highest mean value for the selected character and is chosen as parent of a plus-selected line; S has the lowest mean value for the selected character and is selected as parent of a minus-selected line; and I, with a mean as nearly intermediate between those of L and S as possible, is used as parent of an unselected intermediate line; D, the plates poured from cultures L, S, and I; and E, the daughter cultures grown from spores isolated from the plates in D. L and S are again selected as parents of the plus and minus lines, respectively, and I is carried on without selection.

All the plates from the three different cultures were poured on the same day, and from agar of the same lot. All the spore transfers were made on the same day and all were kept in the same rack under the same environmental conditions. Whatever the influences of environment were they would act on all the cultures in the same way, and such variations as might occur between cultures would therefore be ascribed to heredity.

Some variation in time of sporulation was always found but no definite correlation was noted between the characters studied and the relative rapidity of development. It was found that the difference in size between

TABLE 2

Comparison of spore appendage lengths of early and late spores from the same culture. (Strain 29, experiment 2, generation 17, plus-selected group, culture 3.) Lengths in μ.

LENGTHS	EARLY SPORES	LATE SPORES
9.0	1	2
10.8	1	9
12.6	4	13
14.4	16	14
16.2	16	18
18.0	28	23
19.8	20	12
21.6	8	6
23.4	2	3
25.2	2	
27.0	2	
Total	100	100
Means	18.16±.216	18.03±.234
Standard deviations	3.15±0.15	3.36±0.16
Difference of means	0.13±0.32	

the spores first developed in a culture and those developed several days later was not significant when compared with the differences which were usually found between different cultures.

This fact is shown in table 2 where distributions of measurements of early and late spores from the same culture are given. The early spores were taken when only a few had been formed, the late spores were the last of which the time of formation was definitely known. It should be noted that all the spores measured here, as in all other parts of this investigation, were mature normal spores, being fully septate and having three

dark central cells. The difference between the mean appendage length of early and late spores is $0.13 \pm 0.32\mu$. From SHEPPARD'S tables (PEARSON 1914) it may be found that the chances are fifteen to one that this difference is due to random sampling. Accordingly it may be assumed that the two sets of measurements are parts of the same frequency distribution. Thus it appears that one may safely measure either early or late spores of a culture without fear of introducing serious errors. In practice the cultures were not measured until a vast number of spores had been produced and the sample was then taken by drawing a sterile platinum wire over the fruiting surface of the culture from the bottom of the agar slant to its top. In this way a random sample was doubtless secured.

The sample was now mounted on a clean slide on which a drop of a weak solution of magenta-red in 40 percent alcohol had been placed. The spores were mixed in the solution by stirring with the platinum wire, covered, and the excess of solution removed with blotting paper after which the spores and appendages were clearly defined in the remaining solution. The appendages and the hyaline cells at either end of the spores were stained deeply by the magenta-red, so that both spore length and length of appendage could be measured accurately. The solution did not cause plasmolysis or shrinking of either the spores or the appendages.

Duplicate measurements were avoided by measuring all spores encountered in a path across the slide just below the upper edge of the coverglass and in another such path just above the lower edge of the glass. The chances that any spore was encountered twice were thus rendered exceedingly small.

When the means of the ten cultures of the plus group and those of the minus group had been measured in the manner described above, the cultures with the longest and shortest means were selected to continue the plus and minus selections respectively.

As contrasted with the selection methods of other investigators, it will be seen that only the range of variation happening to occur in a random sample of ten variates is made available by this method. It may sometimes happen that ten variates chosen at random will all fall near the modal condition, but ten is a large enough number so that in general a random sample of this size is likely to spread over a fair proportion of the range of variation. To have made more than ten cultures in each line of descent in each generation would have required an impossible amount of labor in measuring. A larger number, as 100, for instance, would have insured the detection of a greater number of extreme variates, but would also have vastly increased the likelihood of selecting sporadic mutations rather than

extreme plus and minus variates in the range of continuous variation. From one point of view the method might be looked upon as an inefficient one, in that it surely failed to take advantage of the total range of variation in each generation.

However, when it is considered that not only is the culture from which the spore came known to possess the quality for which selection is being made, but the culture derived from each spore in the line of descent is known to possess this quality before that spore is selected as a parent, it is clear that each selection is much more significant than is usual in cases where many individuals are selected in each generation. For example, in the line selected for plus spore length, each spore chosen as a parent was known to be descended from a series of ancestral spores, each of which had the potentiality for producing a longer-spored progeny than nine other spores of its own generation, chosen at random. Similar plans of selection have been used in one or two previous investigations but not extensively nor with so full a knowledge of the behavior of both the ancestors and the progeny of the parent individuals as in the present work.

So far as the writer knows no other investigator of the selection problem has been able to establish the mean of the offspring of each selected individual as was done in this experiment. Most of the organisms which have been used in selection experiments are of such a nature as to prevent the possibility of securing a sufficiently large number of offspring, all of one age and all developed under the same environmental conditions, to establish such means. Obviously to secure as many as a hundred direct offspring from one parent Difflugia, (JENNINGS uses the term parent as applying to the individual of a dividing pair which retains the old shell), one would have to wait for just that many divisions of the parent, which would demand considerable time. The progeny being formed at different times, might be subjected to different environmental influences, so that the range of variation might be greatly increased. It is therefore not possible to know, in such forms as Difflugia, what the mean of the offspring would be were they all produced under the same conditions. When too small a progeny is secured, one does not know whether these approximate the mean value for the generation or lie at one or the other extreme of the range. Pestalozzia is especially favorable in that one can attain a full knowledge of each generation, which is not the case for most forms. Though such data are obtained only by the expenditure of a vast amount of labor it is believed they are well worth while in a serious study of so complicated a problem as that with which we are here concerned.

Experiment 1. Selection for length of spores

In this experiment the character studied was the length of the spore from the tip of the proximal cell to that of the distal cell. In figure 1, frequency polygons for this character are presented for six strains, of which No. 29 was the one used in this experiment. Strain No. 29 was secured from an isolation of fungi from the wood of a sapling of the Para rubber tree, *Hevea brasiliensis*. Previous to its use in this experiment it had been observed for eight consecutive generations in pure culture on Hevea agar. The data concerning these generations are shown in table 3.

The experiment lasted from January 1919 to June 1919 and included ten selections, all of which were made exactly according to the method described above. Through some unfavorable circumstance, one of the selections for the eleventh generation failed to grow and the experiment was thus brought to an end. The entire experiment was done in Sumatra, and Hevea agar was used for growing all the cultures.

TABLE 3

Mean spore lengths of cultures of strain 29, grown prior to the initiation of experiment 1. Measurements in μ.

GENERATION	SPORE LENGTH	RANGE OF MEASUREMENTS
1	23.6	21–29
2	21.7	16–27
3	25.6	21–30
4	22.2	17–26
5	25.1	21–29
6	25.9	23–30
7	25.5	21–30
8	24.8	21–29
9	25.95	25–40
Mean	24.5	

Figure 4 shows the greatest mean spore length in each generation for the plus selections and the least mean spore length in each generation for the minus selection; the so-called intermediate line carried on without selection is shown also. The figure thus shows the range of difference between the plus and minus selections. In case an effect of selection were present, this range should become greater. In this experiment, this is obviously not the case, since the range does not become greater. It is true that it increases at different times for a number of generations, but this increase is followed by a corresponding decrease, so that in the end no permanent

change is effected. The intermediate line shows continual fluctuation from generation to generation. Similar fluctuation has been found by all workers who have made careful studies of variation in pure lines of organisms. It is presumably due to environmental influences, although the writer was unable to correlate very considerable fluctuations in the

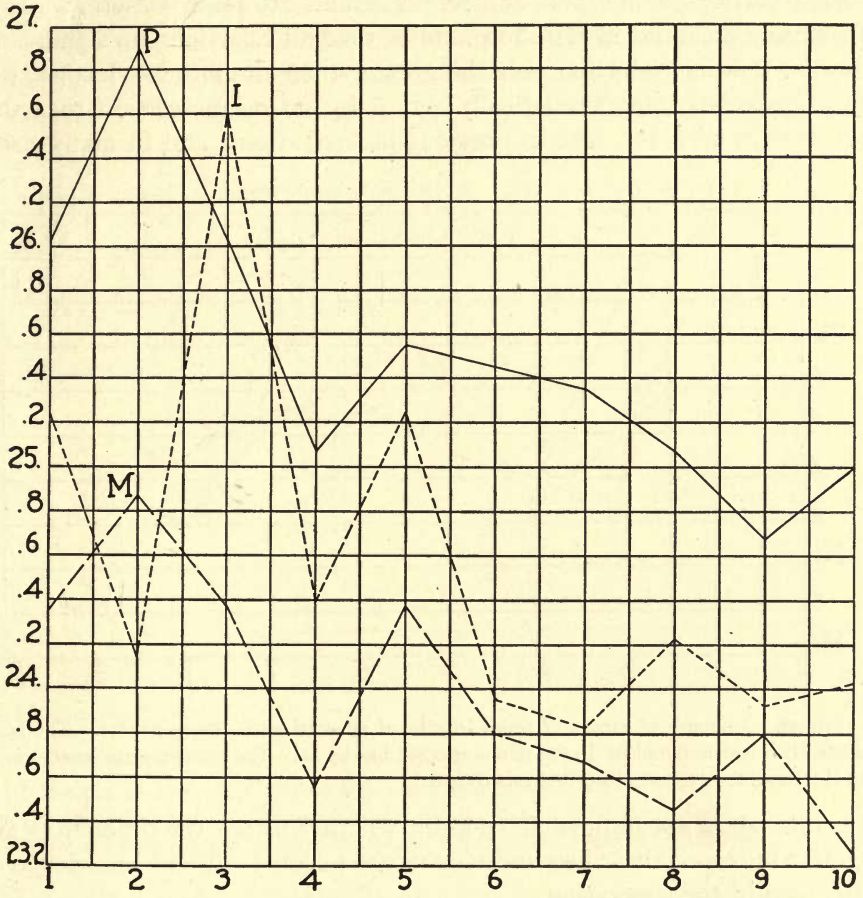

FIGURE 4.—Graph of the mean spore lengths of parent cultures of experiment 1. P represents the parents of the plus-selected line, M those of the minus-selected line, and I, those of the unselected intermediate line. The ordinates are lengths in μ; the abscissae, the generations of the experiment.

spore length of Pestalozzia with any significant fluctuations in any of the environmental factors which are easily measured. The fluctuations in the plus and minus selections appear to be due, not to selection, but to such influences as cause the variations in the unselected intermediate line. In most cases the fluctuations of the plus and the minus selections in a

given generation parallel each other. When this is the case the deviation between the two selections is small. When the fluctuations, for any reason, do not parallel each other the deviations between the two selections may be considerably increased or considerably decreased.

From an inspection of figure 4 it is difficult to tell whether or not the parent cultures of the plus and minus strains are really different. It is absolutely essential in selection studies that all selections be significant, that is, the plus selection must be greater than the minus selection, not only apparently, but statistically. It is by no means certain that this has always been the case in previous investigations, and in many cases

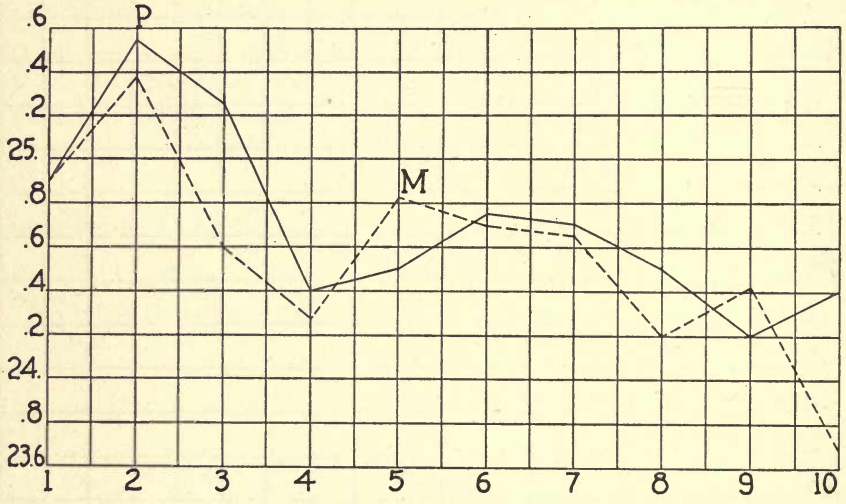

FIGURE 5.—Graph of means of spore lengths of generations in experiment 1. The plus-selected line is designated by P; the minus-selected line by M. The ordinates are lengths in μ; the abscissae, the generations of the experiment.

it is difficult if not impossible to know whether or not the differences are significant, especially where individuals are selected without knowledge of the mean of the generation.

Pestalozzia is a very favorable organism in this respect since one can employ the mean spore length of the selected cultures and know at the same time the means of all the other cultures of that generation, as well as the mean of those means. In this experiment it was found by computing the errors of the differences between the plus and the minus selections that these differences were significant in all cases. The smallest difference found in the course of the experiment, $.900 \pm .206\mu$ in the ninth selection, is more than four times its probable error and may be considered significant.

The means of the different generations of the plus and minus lines are plotted in figure 5 and show variations comparable to those shown in figure 2. If an effect of selection had been secured the means must necessarily have shown increasing deviation one from another throughout the experiment. Such increases as they have shown have been compensated by corresponding decreases so that it becomes evident as the experiment is carried on for a considerable period that these increases and decreases of the deviation between the two selections are only the result of the chance fluctuations which take place within the two lines. It is probable that such

TABLE 4

Mean spore lengths of generations of experiment 1. Each mean was computed from the means of the cultures in its group for that generation. Each mean, except in the intermediate group is accordingly based on approximately 1000 individual measurements. Values are given in μ.

GENERATIONS	PLUS SELECTIONS	MINUS SELECTIONS	INTERMEDIATE GROUP
1 (unselected)	24.91	24.91	24.91
2	25.54	25.38	24.12
3	25.25	24.61	26.59
4	24.41	24.28	24.38
5	24.51	24.82	25.25
6	24.75	24.70	23.94
7	24.71	24.64	23.83
8	24.50	24.21	24.23
9	24.19	24.43	23.92
10	24.41	23.67	24.03
Means of generation means based on means of all cultures in each line	24.727±0.0559	24.622±0.0407	24.552±0.126

Difference between plus-selected line and minus-selected line = 0.105±0.068.
Difference between plus-selected line and intermediate line = 0.18±0.104.
Difference between minus-selected line and intermediate line = 0.07±0.097.

differences would occur between any two separate lines, descended from the same parent but cultivated separately.

The means of each of the generations in the plus-selected line, the minus-selected line, and the unselected intermediate group, and also the mean of means of all cultures in each of these lines for the whole experiment are shown in table 4, while table 12 (in the appendix) gives the means of all cultures measured in this experiment. The differences between the lines are in themselves very small and when compared with their probable errors they are seen to be insignificant. However, had the experiment

been discontinued after three selections it would have appeared that a small, but possibly significant, result had been produced by the selections. The difference between the two lines at that time was 0.267μ, which is probably more than three times its probable error which has not been computed. It is at any rate, more than three times the error of the difference between the plus- and minus-selected lines for the whole experiment.

When selection is continued for a longer time the apparent effect of selection is soon lost and the minus-selected line at times produces longer spores than the plus-selected one. The intermediate form, which is entirely unselected, shows greater fluctuations than either of the other groups, and its mean for the whole experiment is less than that of the minus-selected line. If we assume that the upward and downward swings, so prominent in this experiment, are merely chance fluctuations due to environmental influences we can understand why the intermediate line shows more fluctuation than the others, since it is represented by only one culture in each generation and its generation means are computed from only 100 measurements instead of 1000 as in the case of the other two lines.

Since the two selected lines cannot be shown to differ from one another, or from the unselected line, we can only conclude that selection has been of no avail in producing lines of *Pestalozzia Guepini* distinct for length of spore.

Experiment 2. Selection for length of spore appendages

In this experiment the same strain was used as in experiment 1, namely, No. 29. Table 5 presents the means and ranges of appendage length for the eight generations during which the fungus had been studied prior to the initiation of this experiment, and a frequency polygon of this strain is shown in figure 2.

The plan of the experiment was that explained earlier in this paper and used in experiment 1, and was rigidly followed throughout the whole series of selections which were continued for more than a year. All of the cultures were grown on Hevea agar, made according to the formula already given, from the original lot of Hevea decoction, and used with all the precautions previously mentioned. This work, like that of experiment 1, was all done in Sumatra within a few hundred yards of the place where line No. 29 was originally found.

In this experiment twenty-five selections were made for length of spore appendages, and twenty-five successive generations were grown of an unselected intermediate line also. The appendage lengths of all the parent spores are shown in figure 6. The extreme cultures of the plus and minus

lines parallel each other in a remarkable manner but the differences between them are not great. In one case (generation 11) the two lines are so nearly coincident that parent cultures certainly different for appendage length, could not be secured from the plus and minus lines. The range of variation in appendage lengths did not consistently increase from generation to generation as they should if selection were effective but underwent successive increases and decreases as the two lines drew nearer together or deviated more widely. The differences between the plus-selected parent and the minus-selected parent vary from generation to generation, but are large enough to be of significance except in the one case mentioned.

Figure 7 shows the means of the generations for the whole experiment and from this figure it is at once apparent that the two lines of descent did

TABLE 5

Mean lengths of spore appendages of cultures of strain 29, grown prior to beginning experiment 2. Measurements in μ.

GENERATIONS	LENGTHS OF APPENDAGES	RANGE OF MEASUREMENTS
1	24.5	20–30
2	21.7	10–27
3	23.1	16–30
4	26.7	20–36
5	25.1	20–39
6	22.4	14–39
7	24.0	16–33
8	24.1	19–31
9	21.8	16–40
Mean	23.71	

not become more widely divergent as the number of selections increased. Instead the same upward and downward swings appear as were seen in experiment 1, but in this experiment the two lines parallel each other in these fluctuations in a surprising way.

The means of the generations of each line and the mean of means of each line for the whole experiment are presented in table 6, while table 13 (in the appendix) gives the data for all the cultures grown during the course of the experiment. After examining figure 7 one is not surprised to find that the differences between the three different experimental lines are of no significance. In one case the difference is less than its probable error. Each case where the plus line has grown longer and the minus line shorter has

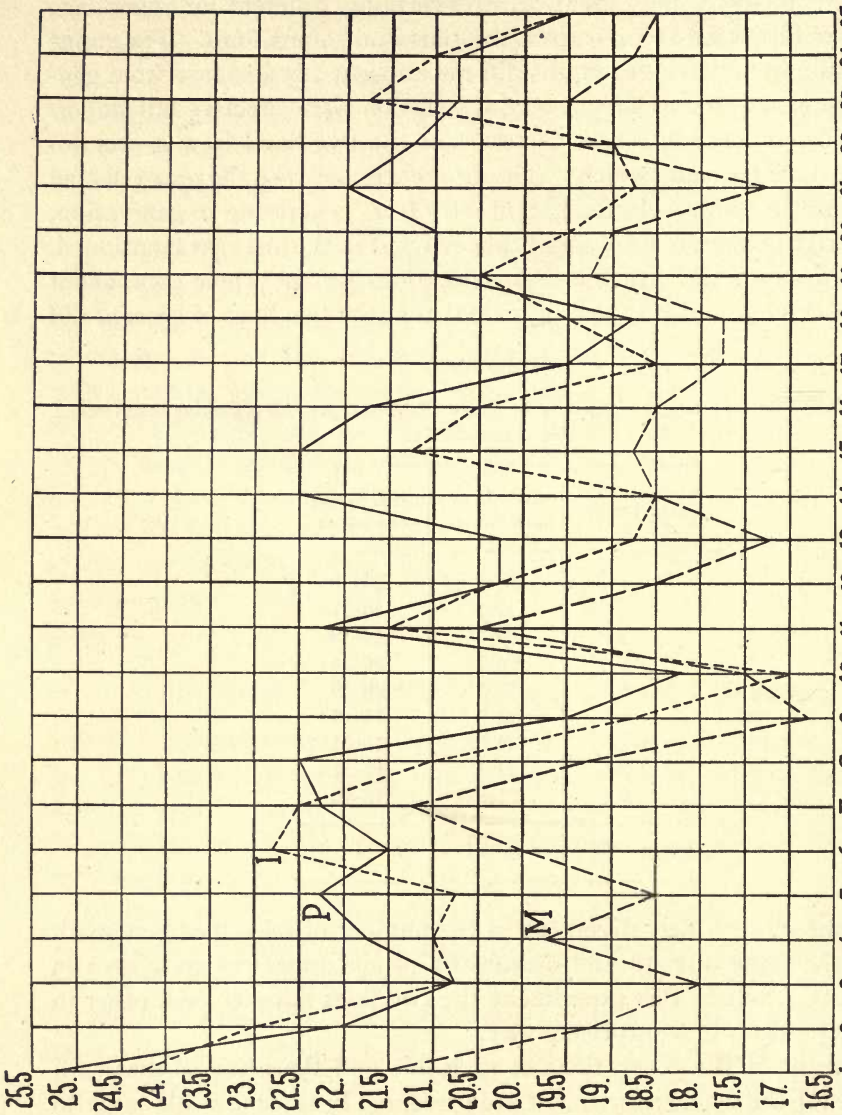

FIGURE 6.—Graph showing the mean lengths of spore appendages of parent cultures of experiment 2. P shows the parents of the plus-selected line; M, those of the minus-selected line; and I, those of the unselected line. The ordinates are lengths in μ; the abscissae, the successive generations of the experiment.

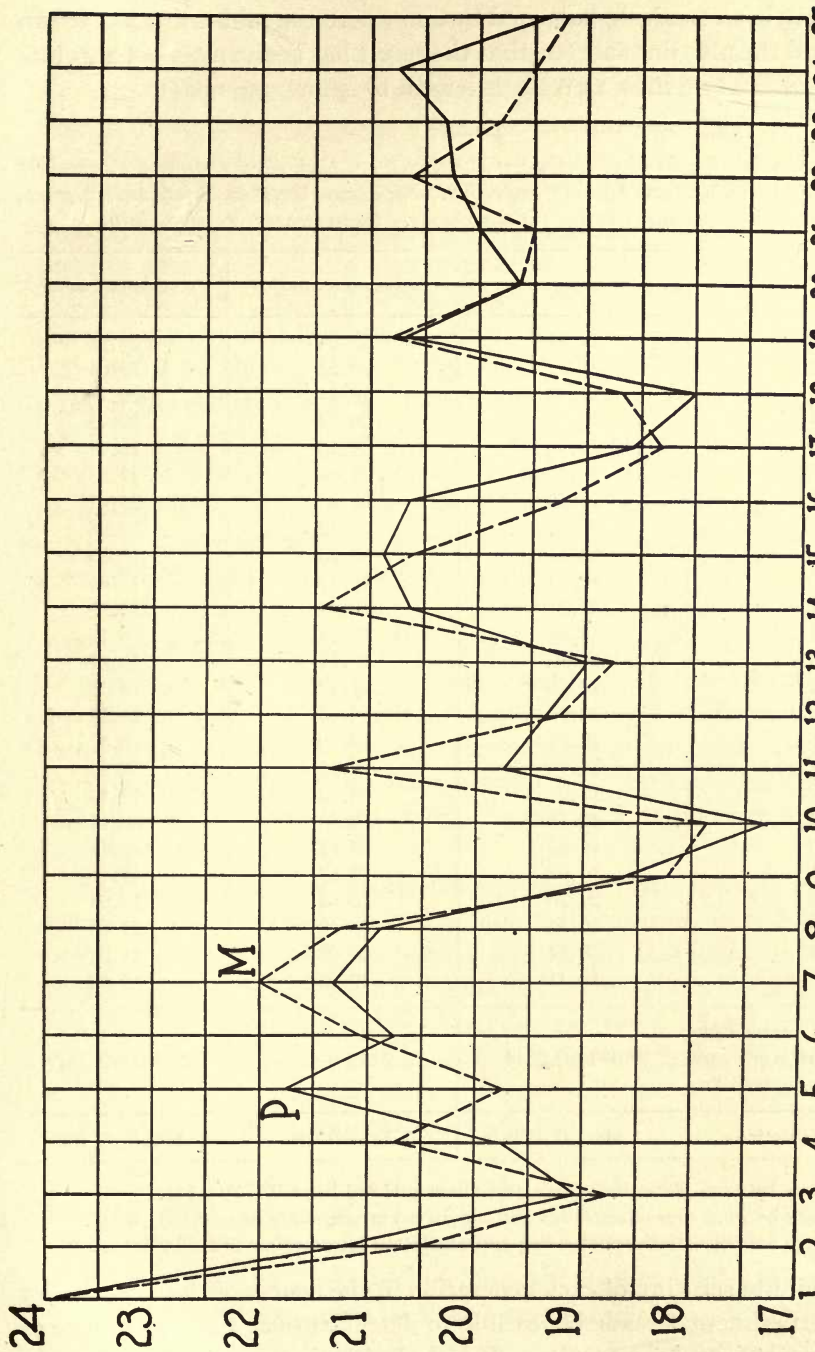

FIGURE 7.—Graph showing the means of spore-appendage lengths of the generations in experiment 2. P represents the plus-selected line; M the group of minus selections. The ordinates are lengths in μ; the abscissae, the generations.

apparently been so nicely balanced by one where the minus line has grown longer and the plus line shorter, that the result has been an almost absolute identity of the two lines as regards length of spore appendages.

TABLE 6

Mean appendage lengths of generations of experiment 2. Each mean was computed from the means of the cultures in its group for that generation. Each mean, except in the intermediate group, is based on approximately 1000 measurements. Measurements are given in μ.

GENERATIONS	PLUS SELECTIONS	MINUS SELECTIONS	INTERMEDIATE GROUP
1	23.94	23.94	23.94
2	21.22	20.54	23.04
3	19.06	18.74	20.78
4	19.66	20.74	20.93
5	21.78	19.73	20.78
6	20.77	21.08	22.63
7	21.32	21.99	22.41
8	20.88	21.22	20.98
9	18.61	18.23	18.83
10	17.33	17.87	17.05
11	19.71	21.37	21.39
12	19.42	19.28	20.14
13	18.99	18.74	18.67
14	20.66	21.42	18.59
15	20.90	20.63	21.24
16	20.61	19.26	20.47
17	18.56	18.34	18.62
18	17.98	18.70	19.44
19	20.68	20.83	20.47
20	19.62	19.62	19.42
21	20.03	19.53	18.65
22	20.20	20.65	19.04
23	20.34	20.29	21.83
24	20.74	20.02	21.06
25	19.31	19.22	19.62
Means of generation means based on means of all cultures in each line	20.394±0.0844	20.223±0.0774	20.502±0.227
Standard deviations	1.908±0.0598	1.746±0.050	1.681±0.160

Difference between plus-selected line and minus-selected line = 0.171±0.115.
Difference between plus-selected line and unselected intermediate line = 0.108±0.242.
Difference between minus-selected line and unselected intermediate line = 0.279±0.239.

The minute scrutiny of each generation in the course of this experiment and of experiment 1 made it possible to detect at once the appearance of any aberrant form by mutation. It is desirable that such control be kept

in all selection studies since it is not improbable that in some cases small mutations have arisen in the course of selection experiments, which have not been detected and have produced effects attributed to selection. In this experiment in the minus line of generation 14, culture 5, a distinct mutation appeared which had a greater length of spore appendages than any other culture encountered in the whole experiment. The spores themselves were also longer, much more slender, and lighter in color than those of any other culture seen during the whole range of my experience with Pestalozzia. This form has since remained distinct through several generations and will be studied further at a later date. It is mentioned here merely to show the possibility that such mutations may account for some of the results which are apparently due to selection. In the present instance the mutation occurred in a plus direction but in the minus line and so could hardly have escaped attention, but had it occurred in the plus line, had it been somewhat less striking, and had it occurred in a less carefully controlled experiment it might have escaped detection, and have greatly confused the results of that experiment.

That the new form was not a contamination from outside instead of a mutation, was made practically certain because no such form had ever been isolated from the surrounding country; because all culture work was carefully done and the usual contaminating organisms were rarely found in the cultures; because Pestalozzia is not a fungus which is likely to be found as an air-borne contamination in cultures; and finally, because blank plates were poured in many generations without ever securing any growth of any organism.

Experiment 2, carried on for more than one year, through twenty-five generations, involving nearly 500 different cultures, and 50,000 spore measurements, should have been a sufficiently thorough and long-continued study to demonstrate any effect of selection which might have appeared. Since no such effect has been demonstrated we can only conclude that the result of this experiment agrees entirely with that of experiment 1 and that it is not possible to produce lines distinct for lengths of spore appendages by even long-continued selection.

Selection of spores according to visible characters

The selection of spores according to their appearance was now undertaken as a check on the results of the previous selections. On account of the small size of the spores of Pestalozzia considerable difficulty was experienced in finding a method by which the measured spores could be

isolated. Finally a special method was devised which has been described in the Botanical Gazette (LaRue 1920).

In this method a selecting device was used which consisted of a brass cone threaded at one end, and turned into a small tube at the other, the end of the wall of the tube being made thin so as to form a sharp cutting edge. The device was screwed into the nosepiece of a microscope in place of one of the objectives. The spores to be examined were mixed in a sufficient dilution in a tube of 1-percent LIEBIG'S beef-extract agar and sterile slides were spread with a thin layer of this agar. When the agar cooled the spores were firmly fixed in a thin transparent agar matrix and could easily be located and measured under the microscope. When a spore of the desired size was located it was carefully centered in the field of vision and the nose-piece of the microscope was turned so as to place the selecting device, of which the tip had just been sterilized by flaming with a gas or alcohol flame, immediately above the spore. In a good microscope, made so that the objectives center properly, this is an entirely mechanical process requiring no skill on the part of the operator. The microscope tube was now lowered until the sharp tube entered the agar and cut a ring completely around the spore. The agar disk containing the spore was now examined to see that only the chosen spore was contained in it, and then lifted with a flattened platinum wire and transferred to a tube of nutrient agar.

The agar used for imbedding the spores on the slides was filtered very carefully to render it as transparent as possible so that it might not interfere with a clear view of the spores. After selection the spores were grown in Hevea agar of the same composition as that used in the former experiments. However, after five selections had been made the cultures were transferred from Sumatra to Michigan, and the supply of Hevea decoction taken with them having been lost in transit, prune-juice agar was used for the remaining cultures of the experiment. The prune agar, which gave satisfactory results, was made by adding 2 percent of agar-agar to a decoction of prunes. Since only one lot was made, and used for all the cultures the exact proportions are of no special significance in the experiment, and are therefore omitted. Care was taken, however, to see that the medium was as uniform as possible for all the cultures.

Experiment 3

The line used for this experiment was No. 17, an isolation made from a leaf-spot of a seedling of *Hevea brasiliensis*, which had been grown in culture and measured for nine generations prior to the initiation of the

experiment. The variation of the strain during these generations may be seen in table 7.

The character selected in this study was length of spore. Spores of the last culture shown in table 7 were mounted in agar on slides and measured. The longest spore found was selected as the parent of a plus-selected line, and the shortest spore encountered was chosen as the parent of a minus-selected line. When sporulation took place in the resultant cultures the spores were measured and the mean spore lengths of the cultures determined precisely as in experiments 1 and 2. Spores from each culture were now mounted and measured, the longest found in the plus cul-

TABLE 7

Mean spore lengths of cultures of strain 17, grown prior to the selections in experiment 3. Measurements in μ.

GENERATIONS	SPORE LENGTHS	RANGE OF MEASUREMENTS
1	22.0	17–24
2	21.1	17–23
3	21.2	19–24
4	23.4	20–29
5	22.3	19–29
6	22.5	17–26
7	23.3	19–27
8	23.5	20–28
9	23.9	20–28
Means	22.58	

ture being selected to continue the plus line, and the shortest found in the minus culture being taken as parent of the next generation of the minus line.

The selections thus begun were continued until ten had been made when the experiment was discontinued. No attempt was made to make the selections in the two lines at the same time. Thus the cultures in the two groups usually developed under somewhat variable environmental conditions so that a given selection is not precisely comparable with the selection of the same number in the other line. However, in the long run this variation is probably not significant since the fluctuations in environment should be about the same in summation for one line as for the other.

From the nature of the method of selection, spores near the extremes of the range of variation were chosen, and accordingly the difference between the selected spore of the minus line, and that for the plus line in a given generation, was always large. The lengths of the selected spores for the experiments are shown in figure 8. The differences between the parent spores of the two groups does not increase significantly during the experiment, nor does it decrease. At the time the cultures were transferred to America the lengths of the parent spores decreased considerably but the deviations between them were unchanged. To what extent the decrease in spore length was due to change of culture medium, and to what extent due to change of climate, is unknown.

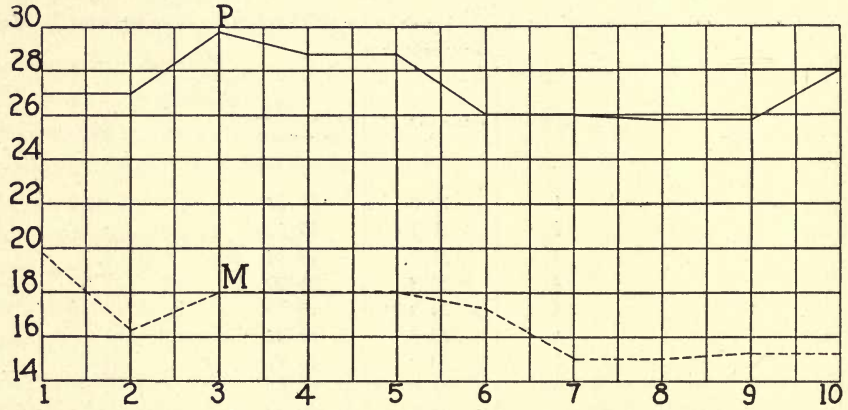

FIGURE 8.—Graphs of the lengths of individual spores selected as parents of the plus and minus line of experiment 3. P, the plus selections; M, the minus selections. The ordinates are lengths in μ; the abscissae, the successive generations.

The mean spore lengths of the generations of this experiment are shown in table 8. The removal of the cultures to America resulted in a decrease in mean spore length which is clearly shown in figure 9. The plus and minus lines did not parallel each other so closely as in experiments 1 and 2. This is to be expected because the selections in the two lines were not usually made at the same time. The two lines were consequently not in harmony in regard to the conditions under which they were developed and their fluctuations were not parallel. In five of the ten generations the minus line has a greater spore length than the plus-selected one. That this result is due to selection is unthinkable. It serves as a warning that small changes, apparently in the direction of a selection effect, should not too readily be considered as due to selection, though they have usually been so interpreted.

The means of means of all the cultures in the two lines when compared are found not to be significantly different. The upward and downward swings have had the same total effect in one line as in the other though the different lines have scarcely paralleled each other at all during these swings. Experiment 3, then, entirely substantiates the results gained in experiments 1 and 2, and shows that the selection of visible characters within a pure line is no more effective in producing distinct groups than selection on the basis of progeny.

In the course of experiment 3 an aberrant form appeared, which seems to be a mutation. This arose in the plus line in the seventeenth generation.

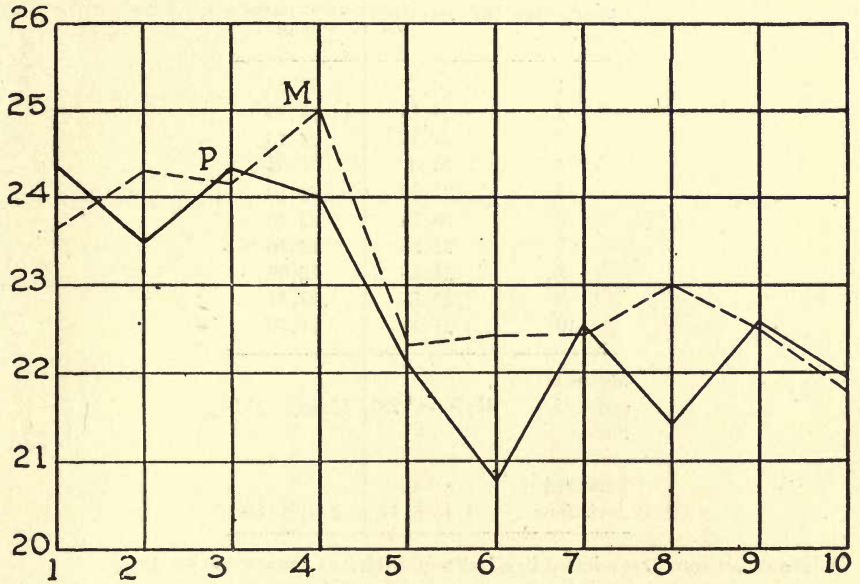

FIGURE 9.—Graphs of the spore lengths of cultures grown in experiment 3. P shows the cultures of the plus line; M those of the minus line. The ordinates are lengths in μ; the abscissae, the generations of the experiment.

In regard to length of spores and of spore appendages it is identical with strain 17 from which it arose. However, it has vegetative characters quite distinct from those of strain 17 and this makes it appear very different to the naked eye. Strain 17 produces a very small amount of mycelium on the surface of the agar substratum, which becomes almost completely covered with a black, slimy mass of spores. The new form produces a thick felt of mycelium all over the surface of the agar, and sporulation is much more tardy than in strain 17. The spores are produced in small black masses which are rather sparsely scattered over the surface of the

mycelium. Thus far the form has remained distinct for two generations, and will be studied further in the near future. If it continues to show distinctive characters it will have to be considered as a mutation, though one which affected fewer characters than the one encountered in experiment 1. More detailed statements concerning both these mutations will be presented in another publication.

TABLE 8

Mean spore lengths of cultures grown in experiment 3. One culture was grown in each line in each generation. Each mean is based on 100 measurements. Measurements in μ.

GENERATIONS	PLUS SELECTIONS	MINUS SELECTIONS
1	24.37	23.65
2	23.53	24.30
3	24.34	24.16
4	24.01	25.04
5	22.09	22.30
6	20.78	22.40
7	22.56	22.46
8	21.43	22.98
9	22.58	22.51
10	21.97	21.76
Means of generation means	22.766±.251	23.16±.212
Standard deviations	1.18±.17	1.01±.15

Difference between plus-selected line and minus-selected line = 0.390±0.328μ.

Experiment 4

It was originally intended that this experiment should be carried on for an extended period of time. In order to lessen the labor required, some changes were made in the method which had been used in experiment 3. The spores were selected and grown in the same manner as in experiment 3 but the cultures of each generation were not measured. Instead it was planned to measure them only from time to time to determine whether or not the plus and minus lines were diverging one from another. Unfortunately only a few selections had been made before the writer left Sumatra. The strain used, No. 5, which was isolated from a leaf spot of cocoanut palm and had been successfully grown for nine generations in

culture with the results shown in figures 1 and 2, and in table 9, did not thrive well in Michigan, even when kept in a constant-temperature room, so after six selections the experiment was discontinued. While the experiment included fewer selections than is desirable, a large number, perhaps the majority, of selection experiments have been carried no farther, and no apology need be made for presenting the results. These are shown in table 10 and the measurements of the selected parent spores are presented in figure 10.

The spores selected, on the basis of spore length, as parents of the plus and the minus lines, were significantly different from each other in every generation, so that there can be no doubt that real selections were made.

TABLE 9

Mean spore lengths of cultures of strain 5, grown prior to the initiation of experiment 4. Measurements in μ.

GENERATIONS	SPORE LENGTHS	RANGE OF MEASUREMENTS
1	24.4	20–29
2	21.1	17–26
3	22.3	17–24
4	20.2	17–24
5	22.2	19–26
6	22.4	19–29
7	20.6	17–24
8	23.2	19–29
9	21.9	18–25
10	21.5	17–27
Mean	21.98	

The cultures were removed from Sumatra after three minus selections and two plus selections had been made. The fourth, fifth, and sixth selections of the plus line show no decrease in spore size following this change of climate, since they are all at least equal in size to the third selection, and the sixth selected spore equals the size of the first and second parent spores of the line. In the minus line, however, the third, fourth, and fifth selections show a decided decrease in spore size. The sixth selected spore is equal in size to the second, but it should be noted that another chosen spore of precisely the same size as the fifth selected spore failed to grow. It is rather uncertain whether any of these changes may be attributed to climatic changes.

Only the cultures of the fifth and sixth generations were measured. In the fifth generation the culture of the plus line had a greater mean spore length than that of the minus line, but in the next generation the result was reversed, and the culture of the minus line had the higher mean. The summation of the two generations shows that the two lines are not distinct, the difference between them being barely larger than its probable error. The result of this experiment is the same as that for the three preceding experiments, namely, that selection has no appreciable result.

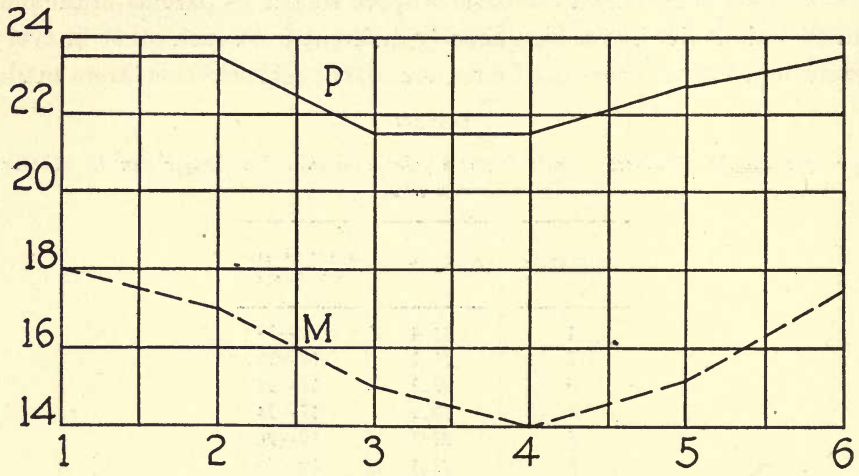

FIGURE 10.—Graphs showing the lengths of the individual spores selected as parents of the plus and minus lines of experiment 4. P indicates the plus selections; M, the minus selections. Lengths in μ are given as ordinates; the successive generations as abscissae.

The evidence gained from two experiments, in which visible characters were selected, is in entire agreement with that secured from the more extensive experiments in selection according to progeny, and all the experiments, whatever the method and strain used, consistently show that selection within pure lines of Pestalozzia is entirely ineffective, but that rarely mutations occur which are significantly different from the parent line.

DISCUSSION

JENNINGS (1916, 1920) considers that if the experiments which have been supposed to demonstrate the negative result of selection had dealt with characters which were less likely to be influenced by degree of maturity and environmental influences, had been more carefully conducted, and had involved a larger number of selections, they would likely have shown a

positive result. The Pestalozzia investigation has greater evidential value. It was carried on for 10, 25, 10, and 6 generations, respectively, in the several experiments. The characters studied were such as to admit of certainty as to maturity of the observed individuals, and they were not more subject to environmental influence than those considered in investigations which have seemed to give evidence of an effect of selection. In certain respects, which have already been pointed out, the different generations have been more fully known, and a larger number of variates have been considered, than in any other investigation yet reported. Nevertheless, the different experiments consistently fail to show any effect of selection. The evidence secured in this study then is a direct confirmation of the results of JOHANNSEN and the other early workers on the pure-line problem. In attempting an explanation of the discrepancy between these results and those which contradict them, it is necessary to attempt to evaluate the experiments that have shown a positive selection effect.

TABLE 10

Means of generations measured in experiment 4. Measurements in μ.

GENERATION	PLUS-SELECTED LINE	MINUS-SELECTED LINE
5	21.142±.110	20.802±.117
6	20.428±.114	21.500±.112
Means	20.785±.241	21.151±.236

Difference between plus-selected line and minus-selected line = 0.366±0.337μ.

The results of STOCKING (1915), while sufficiently striking and credible, can be given little weight in a general consideration since the abnormal characters dealt with so strongly suggest a pathological condition of the organism. To be given weight in a discussion of selection as a factor in evolution, the supposed modification produced by selection must affect a normal character in a direction shown by existing forms in nature to have been actually traversed by these other forms in the course of their evolution.

MIDDLETON (1915) found selection for fission rate in Stylonychia effective to a degree which must have been surprising, even to believers in the possibility of modification by selection. The great readiness with which organisms could be altered in MIDDLETON'S experiments indicates that the response of Stylonychia to selection is to some degree exceptional. ROOT (1918) suggests that "the inheritance of variations in fission rate in Sty-

lonychia might be due to the accumulation of waste products in the cytoplasm of the slowly-dividing, large-sized group." The fact that MAST (1917) secured two lines with different fission rates in *Didynium nasutum* without selection is suggestive that mutation may offer an alternate explanation of MIDDLETON'S results.

ROOT (1918) working on Centropyxis has secured data worthy of consideration. In one experiment, using mass selection, he found a decided effect of selection on the number of spines after only four selections. However, the number of individuals studied was very small,—88 in the high series and 77 in the low series. In another mass selection the results were exactly opposed to those of the first; that is, the parents with a low spine number produced offspring with more spines than those from parents with a large number of spines. The latter result obviously cannot be due to selection, though had it stood alone it might have been so interpreted. The two experiments show the danger of drawing conclusions from a small number of individuals and a few selections.

In a third experiment in which individuals were selected according to the character of their progeny no considerable effect was gained until the fourth selection. From the fourth selection a large effect was secured. One selected individual gave offspring all of which had a high spine number. This individual (5aIa of ROOT'S series), the offspring of which are responsible for a decided increase in the number of spines in the plus series, may have been a mutation. The number of spines in the low series did not appreciably decrease during the selection period. The whole experiment included only a small number of individuals, 56 in the plus series and 51 in the minus, and only four selections were made.

In the fourth experiment in which mass selection for number of spines was practiced, two populations were segregated which varied in respect to spine number. The difference between the two populations was small and fluctuated from generation to generation. In the second selection period the difference was less than in the preceding period. The number of individuals observed was larger than in the other experiments but still relatively small. It would have been interesting to see what the result of a continuation of the cultures would have been. As it is, one suspects the separation to have been only a temporary one, such as is shown for Pestalozzia in table 4 of this paper.

HEGNER (1919) carried on extensive selection experiments on Arcella and finds that it is possible to separate by selection within families groups which are distinct in regard to the selected character. In his principal experiment, however, the effects of selection on number of spines are by no

means consistent. The first set of selections produced a higher number of spines in the group selected for low spine number than in the group selected for high spine number. It is unfortunate that at this point a change was made in the method of selection. The author attributes the effect secured in further selections to the use of a better criterion for the selection. The effect may have been due to independent chance fluctuations within the two groups and not to selection at all but the change in procedure prevents this being detected. It would have been very interesting to know whether or not such divergences would have occurred had the original method been continued. Five further selection periods showed an increase in spine number in the group selected for high spine number and a decrease in the group selected for low number. The difference between the high and the low series, however, does not show a steady increase, but fluctuates from one selection period to the next. When the divergence between the two groups was at a maximum, selection was stopped, so that we do not know whether the divergence would have decreased or not had selection been continued.

On the cessation of selection the divergence between the groups decreases very little for one period. In the next period the divergence falls to almost nothing while in the next following period it again rises to nearly half that secured at the end of the six selection periods. It is approximately equal to that found in the second selection period. In the second selection period the increase in divergence is attributed to selection. To what is it due in this non-selection period? It seems logical to assume that it is due to the same cause in both cases, probably to the independent chance fluctuations of the two groups.

Further selection for one period in the high line resulted in a slightly larger number of spines in the group selected for low spine number than in that selected for high spine number. That this is due to selection is, of course, impossible.

Three selection periods were effective in that they developed two divergent lines within the low line established by the early selection. Here again the divergence fluctuates from period to period. After selection ceases the divergence becomes greater than it was during the selections. This again indicates that the divergence may be totally unrelated to the selection. In experiment 1 of this paper may be found similar divergences, which were only temporary.

In another experiment HEGNER practiced selection for diameter and spine number for a period of nine days. During this time distinct high and low lines were produced. In a non-selection period of thirteen days following the selections the divergence between the high and low series

continued to increase to a marked degree. Obviously this continued increase was not due to selection; on the contrary one would expect a lessened divergence between the high and low series following the cessation of selection. The increased divergence during the non-selection period was almost entirely due to increase in size and spine number in the high series. That this series later produced some very large forms, and that great difficulty was experienced in securing offspring from this group are facts that may be indicative of abnormality in the group. As in HEGNER'S other experiments, relatively few individuals were studied.

The extensive and painstaking studies of JENNINGS (1916) on Difflugia present what appears to be the least questionable evidence of the effectiveness of selection yet published. In one experiment he made selections for seven periods, each of which, except the first, included one generation. Selection was made for number of spines, individuals with from 1 to 3 spines being chosen as parents for a "low" set, and those with from 5 to 8 spines being retained as parents of a "high" set.

The selection was apparently effective and is so interpreted by JENNINGS. Two lines were formed by the selection which differ in mean spine length. However, a closer examination shows that the divergence of the two lines varied from generation to generation. In the second period the divergence is equal to nearly a whole spine (.95 spines), but in the third period the divergence falls to .03 spines, which means the lines are really identical statistically. From the third period on the divergence continually rises and falls but never again reaches so high a point as in the second period. If the divergence is due to selection why is the effect so great in the second period, and why do further selections result in a decreased divergence?

The individual measurements are not published, but if we compute the error of the difference between the means of the two groups, using the generation means, we find it to be .103. The difference between the means of the two groups is $.22 \pm .103$ spines. The divergence is barely more than twice its probable error and so is not statistically significant.

During the selection periods the two groups showed fluctuations in regard to diameter of shell and length of spine which are similar to those already noted for number of spines. Table 11 shows the divergence of the two groups for each of the three characters.

It may be seen at a glance that the differences do not steadily increase with an increased number of selections but rise and fall as if by chance. The difference in spine number is greatest in the second period, that of shell diameter is greatest in the fourth period; and that for spine length

is greatest in the fifth period, falling to a minus value in the sixth period. The mean for shell diameter is the only one which is equal to three times its probable error. Apparently the two lines are not demonstrably distinct.

In family 314 also, JENNINGS attempted to isolate lines distinct for spine number. Selection was continued for seven periods in the same way as within family 304. The means of the "high"-selected line and of the "low"-selected line were separately computed for each generation. The differences between the two lines varies from generation to generation as in family 304. It is noteworthy that the greatest divergence (1.24 spines) appears in the first selection period; the lowest deviation in the sixth period. The difference between the mean numbers of spines for the two groups is

TABLE 11

Differences between "low" and "high" selected groups of family 303 of Difflugia.

SELECTION PERIODS	DIFFERENCES IN NUMBER OF SPINES	DIFFERENCES IN SHELL DIAMETER IN UNITS OF SCALE	DIFFERENCES IN LENGTH OF LONGEST SPINES IN SCALE UNITS
1	.41	.52	.48
2	.95	.63	.57
3	.03	1.16	.39
4	.35	2.88	.02
5	.25	.61	.67
6	.34	.95	.29
7	.60	.94	.13
Mean	.22±.103	.93±.26	.76±.299

.42±.25 spines. Since the divergence is less than twice its probable error, we can only conclude that the lines are not distinct and that the apparent difference is due to the independent fluctuation of the two groups.

In another and more extensive experiment, JENNINGS sought to isolate distinct lines by selection within family 326. Again spine number was the character selected, and selection was continued for twelve periods. For the first six periods selection was ineffective and both plus and minus divergences between the "low" and the "high" groups appeared.

As in one of HEGNER'S experiments, a change in the method of selection, made at this time, prevents us from knowing what would have resulted from further selections in which large numbers of individuals were used. Following the change in method two series distinct for spine number were

isolated by selection during six periods. The difference between the means is 0.51 ± 0.109 spines or less than five times its probable error.

During the six selection periods following the change in the method of selection (periods 11 to 16) the divergence between the two groups did not constantly increase but fluctuated from period to period. In the eleventh period it was .52 spines, in the twelfth it fell to .49 spines, in the thirteenth it rose to .84, the highest point reached. In the fourteenth period the difference fell to .28 spines, in the fifteenth to .23 spines, which is probably not significant, and in the sixteenth or last period it again rose to .66 spines. If the divergence in period thirteen is due to selection, it is difficult to see why further selection for the same number of periods that caused this divergence, should cause a decrease in the difference of the two groups.

After selection was discontinued the two groups were kept under observation during five different periods which varied from eight to twenty-two days in length. The mean spine number of the individuals in the low-selected group was 5.19 while that of the high-selected group was 5.58. The error of the difference between the means of the two lines is 0.178 spines while the difference is only 0.39 spines, or slightly more than twice its probable error. The difference would not be considered significant if it were not for the fact that the variations in the various lines, though great, are insufficient to bridge the gap between oppositely selected lines. Is it a fair criticism that Difflugia is insufficiently plastic to show rapid changes from a certain condition of the shell after that condition is once attained? The results suggest that the shell itself plays a part in determining the character of the new individual formed by division of the old one. This shell effect may be regarded as superposed upon the other factors determining the form of the new shell. Such an effect might be expected upon physical grounds. Naked protoplasm protruded from a large orifice is under different surface conditions than if the orifice were smaller; the extruded protoplasm would be expected to assume a different form and size in the two cases, and the newly secreted shell would therefore be different. In other words, any accidental size modification might be maintained through several generations, not through any protoplasmic modification, but merely because the nature of the shell imposes certain physical conditions upon the development of the new individuals. A modification having once occurred in the shell through any cause, becomes comparable to an environmental factor which is maintained for several successive generations. The nature of new individuals is largely determined by the environment, and of the environmental factors the shell is one of the chief. If this

suggestion has any validity, it follows that the two groups, while apparently different, were not significantly different, even while selection was being practiced.

If two lines are isolated within a strain and cultivated separately these lines will be independently subjected to the environmental influences which cause variation for the character studied. The lines will tend to coincide if they are subjected to these influences at the same time and to the same degree. This is the case with the strain of Pestalozzia studied in the author's experiment 2 and is shown in figure 7. It is apparent that there is a certain rhythm, either in the organism itself or in the environmental factors, which causes upward and downward swings of the organism in respect to the character under observation. Since great care was taken to have the two groups subject to identical conditions at all times the two groups follow one another very closely in these swings. In experiment 1 the swings follow each other less closely but with some exceptions there is a strong similarity of behavior in the two lines as is shown in figure 5. In MAST's work with Didynium, where two groups have been separated, apparently by a mutation, these groups fluctuate in the same direction at the same time, with remarkable unison.

If two lines are isolated and cultivated separately, but the offspring are produced in such a way that those of the two lines are subjected to the environmental factors causing variation for a given character, at a different time, or in a different degree, each of the lines will fluctuate in a different way, and this will cause divergence between the two. However if the lines are continued the fluctuations will compensate one another so that in the end the two lines will be found to give practically the same mean value for the character studied.

To the writer it appears that JENNINGS's results with Difflugia, ROOT's with Centropyxis, and HEGNER's with Arcella, are explained by the above assumption quite as well as are his own with Pestalozzia. If this be true they tend rather to demonstrate the ineffectiveness of selection within pure lines than the opposite.

It is warranted to express the belief that the "pure-line" hypothesis is still valid. The writer does not wish to express an opinion that ultimately the effectiveness of selection may not be shown, but he feels that the burden of proof lies with those who question the results of JOHANNSEN and his followers. At the present time incontestable evidence of an effect of selection is wanting. From the recent investigations made in this field it is obvious that the situation is vastly more complicated than was formerly supposed. The truth or falsity of the "pure-line" hypothesis

will be finally determined by numerous investigations with various organisms, involving infinite care and toil. There is need of more investigations of the type which JENNINGS has made on Paramecium and Difflugia, but carried for longer periods, with organisms more favorable for study, if they may be found.

Nothing has been said in this paper, of the bearing of the "pure-line" hypothesis on theories of evolution. Almost every worker who has made careful biometric studies of highly variable groups has recorded the occurrence of mutations or "something like mutations." JENNINGS, ROOT, MAST, and HEGNER have all recorded them for the lower animals. BARBER (1907) noted the occurrence of mutation in yeasts; STEVENS (1920) has reported a number of mutations in Helminthosporium; and the writer has found mutations in Pestalozzia. It is unnecessary to catalogue the numerous cases of mutation recorded in various groups of higher plants and animals. Further careful study will doubtless reveal many more. When more is known of their occurrence, frequency, and reaction to environment, to other existing organisms, and to one another, may we not be able to conceive of evolution, even without cumulative growth of characters by the selection of fluctuating variations?

CONCLUSIONS

Selections according to progeny within pure strains of Pestalozzia were made for length of spores for ten generations, and for length of spore appendages for twenty-five generations, without result. Selections according to visible characters were made for spore length for ten generations in one experiment and for six generations in another, without establishing differences between the plus-selected and minus-selected lines. Whatever method and strain may be employed, selection is totally ineffective in establishing distinct lines within pure strains of *Pestalozzia Guepini*.

Mutations which give rise to lines significantly different from the parent lines, infrequently occur.

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APPENDIX

Tables of constants of experiments 1 and 2

TABLE 12

Means and standard deviations of spore lengths of cultures grown in experiment 1. The mean of each culture was calculated from at least 100 measurements. Values are given in units of the scale of the eye-piece micrometer used. 1 unit = 1.8 microns. Single spore culture of parent strain (No. 29). Mean = 13.80, $\sigma = 1.58$.

Generation 1 of experiment

CULTURE	MEAN	σ	CULTURE	MEAN	σ
1	14.46	1.55	11	14.16	1.43
2	13.90	1.76	12	13.72	1.82
3	13.61	1.85	13	13.64	1.43
4	13.97	1.63	14	13.62	1.54
5	13.51	1.79	15	14.18	1.84
6	14.02	1.90	16	13.58	1.67
7	13.71	1.80	17	13.85	1.87
8	13.57	1.71	18	13.79	1.82
9	13.95	1.47	19	13.95	1.65
10	13.71	1.71	20	13.89	1.52

Mean of generation 13.84.

Generation 2

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	14.03	1.41	14.23	1.66	13.40	1.10
2	14.29	1.44	13.99	1.38		
3	14.94	1.52	14.19	1.39		
4	13.95	1.43	14.00	1.29		
5	14.33	1.49	13.82	1.39		
6	14.35	1.52	14.00	1.27		
7	14.94	1.55	14.31	1.43		
8	13.43	1.33	14.12	1.52		
9	13.72	1.54	14.17	1.62		
10	13.94	1.93	14.15	1.33		
Generation means	14.19		14.10		13.40	

TABLE 12 (continued)

Generation 3

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	14.46	1.26	13.55	1.24	14.77	1.38
2	13.88	1.28	13.92	1.22		
3	14.19	1.21	13.60	1.38		
4	14.11	1.09	13.61	1.29		
5	14.34	1.25	13.60	0.96		
6	13.75	1.18	13.54	1.18		
7	13.75	1.16	13.79	1.33		
8	14.22	1.33	13.77	1.21		
9	13.74	1.07	13.59	1.33		
10	13.88	1.15	13.70	1.31		
Generation means	14.03		13.67		14.77	

Generation 4

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.50	1.17	13.27	1.13	13.55	1.14
2	13.62	1.22	13.68	1.24		
3	13.87	1.04	13.63	1.32		
4	13.11	1.12	13.79	1.13		
5	13.37	1.11	13.32	0.99		
6	13.93	1.33	13.09	1.29		
7	13.78	1.12	13.64	1.07		
8	13.53	1.37	13.44	1.29		
9	13.27	1.01	13.55	0.95		
10	13.59	1.21	13.52	1.07		
Generation means	13.56		13.49		13.55	

TABLE 12 (continued)

Generation 5

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.92	1.17	13.93	1.12	14.03	1.32
2	13.55	1.04	14.19	1.26		
3	14.07	1.31	13.43	1.29		
4	13.60	1.24	13.75	1.13		
5	13.77	1.02	13.41	1.21		
6	13.88	1.10	13.71	1.42		
7	13.40	1.19	13.81	1.10		
8	13.47	1.37	14.14	1.27		
9	14.05	1.02	13.53	1.12		
10	13.63	1.10	14.04	1.19		
Generation means	13.73		13.79		14.03	

Generation 6

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.73	1.17	13.75	1.18	13.31	1.12
2	13.74	1.18	13.65	1.19		
3	13.91	1.12	13.75	1.33		
4	14.14	1.01	13.60	0.88		
5	13.62	1.05	13.23	1.08		
6	13.65	1.00	13.87	1.06		
7	13.64	1.11	13.68	1.18		
8	13.69	1.10	14.10	1.13		
9	13.84	1.20	13.66	1.36		
10	13.50	1.15	13.91	1.24		
Generation means	13.75		13.72		13.31	

TABLE 12 (continued)

Generation 7

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.84	1.28			13.26	1.06
2	13.76	1.21				
3	13.59	1.13	14.17	1.40		
4	13.78	1.09				
5	13.33	1.33				
6	14.08	0.98				
7	13.86	1.12	13.76	1.33		
8	13.45	0.97	13.67	1.37		
9	13.87	1.22	13.17	1.66		
10	13.75	1.22				
Generation means	13.73		13.69		13.26	

Generation 8

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.46	0.95			13.47	0.98
2	13.65	1.11				
3	13.79	1.17	13.03	1.01		
4	13.93	1.06	13.79	1.05		
5	13.89	1.07	13.49	0.98		
6	13.18	1.11	13.60	1.15		
7	13.42	1.05	13.59	1.13		
8	13.80	1.14	13.17	1.08		
9	13.47	1.08				
10	13.49	1.15				
Generation means	13.61		13.45		13.47	

TABLE 12 (continued)

Generation 9

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.46	1.23	13.43	1.07	13.29	1.03
2	13.50	1.17	13.56	1.21		
3	13.28	1.08	13.92	1.03		
4	13.71	1.13	13.84	1.02		
5	13.26	1.06	13.57	0.99		
6	13.53	1.09	13.21	1.34		
7	13.55	1.31	13.47	1.11		
8	13.45	1.04	13.68	1.05		
9	13.38	1.17	13.25	0.98		
10	13.31	1.08	13.75	1.08		
Generation means	13.44		13.57		13.29	

Generation 10

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.45	1.22	13.05	1.03	13.35	1.03
2	13.31	1.15	13.13	1.38		
3	13.89	1.33	13.14	1.00		
4	13.03	0.95	12.96	1.02		
5	13.71	0.94	13.27	0.91		
6	13.86	0.96	13.26	0.88		
7	13.67	0.99	13.19	1.18		
8			13.12	1.08		
9	13.57	1.11	12.92	1.17		
10	13.54	1.04	13.44	1.07		
Generation means	13.56		13.15		13.35	

Means of all cultures in experiment 1

PLUS SELECTIONS	MINUS SELECTIONS	INTERMEDIATE GROUP
13.736 ± 0.0311	13.679 ± 0.0226	13.640 ± 0.070
$\sigma = 0.435 \pm 0.0219$	$\sigma = 0.331 \pm 0.0159$	$\sigma = 0.326 \pm 0.0492$

Difference between plus selections and minus selections $= 0.057 \pm 0.038$
Difference between plus selections and intermediate group $= 0.10 \pm 0.058$
Difference between minus selections and intermediate group $= 0.039 \pm 0.054$.

TABLE 13

Means and standard deviations of appendage lengths of cultures studied in experiment 2. The mean of each culture was calculated from 100 or more measurements. Values are given in units of micrometer scale: 1 unit = 1.8 microns. Single-spore culture of parent strain (No. 29). Mean = 12.42, σ = 2.08.

Generation 1 of experiment

CULTURE	MEAN	σ	CULTURE	MEAN	σ
1	12.67	2.20	11	13.62	2.21
2	12.50	2.22	12	13.52	3.07
3	13.58	2.30	13	13.69	2.96
4	13.57	2.40	14	13.48	2.55
5	13.85	2.43	15	12.48	2.54
6	13.46	2.26	16	14.01	2.52
7	13.64	2.52	17	12.01	2.81
8	13.10	2.30	18	13.08	2.42
9	13.66	2.34	19	13.74	2.72
10	13.81	2.29	20	12.46	2.33

Mean of generation 13.30

Generation 2

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.70	2.00	11.59	2.17	12.80	2.28
2	12.16	1.98	11.42	2.24		
3	12.09	1.95	11.60	2.31		
4	12.03	1.88	11.44	2.07		
5	11.92	2.26	11.40	2.06		
6	11.44	2.19	10.63	1.91		
7	11.49	2.03	11.36	2.22		
8	11.92	2.71	11.60	2.13		
9	11.14	2.16	11.68	2.29		
10	11.97	1.98	11.42	2.03		
Generation means	11.79		11.41		12.80	

TABLE 13 (continued)

Generation 3

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.05	1.77	10.31	2.34	11.55	2.33
2	9.99	2.20	10.41	1.67		
3	9.84	2.07	10.44	2.20		
4	11.29	2.17	11.01	2.09		
5	10.64	2.16	10.78	2.19		
6	10.85	2.08	10.17	1.60		
7	10.48	2.12	10.01	2.04		
8	10.53	2.07	10.11	1.76		
9	10.75	1.96	10.49	2.27		
10	11.52	1.99	10.41	2.10		
Generation means	10.59		10.41		11.55	

Generation 4

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.60	1.88	12.42	2.08	11.60	2.08
2	9.99	2.16	11.69	2.60		
3	11.04	1.83	10.97	1.98		
4	10.61	2.18	11.32	1.83		
5	10.18	1.94	11.78	2.36		
6	11.48	2.18	11.23	2.43		
7	12.14	2.17	10.99	2.03		
8	11.40	2.20	11.72	2.27		
9	11.18	1.77	11.82	2.36		
10	10.55	1.93	11.22	2.17		
Generation means	10.92		11.52		11.60	

TABLE 13 (continued)

Generation 5

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.82	2.46	11.93	1.96	11.55	2.43
2	12.22	2.08	10.95	2.44		
3	11.60	1.98	10.51	2.31		
4	12.42	2.38	11.13	2.67		
5	11.64	2.17	10.54	1.96		
6	12.33	2.12	11.52	2.14		
7	12.39	2.22	11.17	2.35		
8	12.19	2.30	10.26	2.46		
9	12.04	2.43	11.15	2.28		
10	12.33	2.63	10.45	2.37		
Generation means	12.10		10.96		11.55	

Generation 6

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.96	2.57	11.79	2.36	12.57	2.34
2	11.92	2.36	11.47	2.31		
3	11.91	2.13	11.39	2.30		
4	11.04	2.31	12.40	2.21		
5	11.60	2.16	12.00	2.37		
6	11.50	1.90	11.08	2.18		
7	11.80	2.60	11.88	2.41		
8	11.58	1.86	11.82	2.47		
9	11.19	2.61	11.20	2.31		
10	11.93	2.61	12.02	2.84		
Generation means	11.54		11.71		12.57	

TABLE 13 (continued)

Generation 7

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	12.11	2.16	11.86	2.34	12.45	2.30
2	12.34	1.98				
3	12.08	2.25	12.29	2.58		
4	12.10	2.27	12.36	2.22		
5			12.49	2.74		
6	12.16	2.35	12.14	2.15		
7	11.87	2.31	11.94	2.25		
8	12.38	1.86	12.35	2.44		
9	12.25	2.13	12.25	2.10		
10	12.30	1.65	11.82	2.20		
Generation means	12.18		12.16		12.45	

Generation 8

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.58	1.99	11.84	2.09	11.65	2.18
2	11.72	1.96	11.91	2.20		
3	12.47	1.94	10.68	1.74		
4	11.41	2.26	11.92	2.05		
5	11.42	2.12	12.08	2.14		
6	11.71	2.17	12.03	2.09		
7	11.51	2.22	12.34	2.02		
8	11.39	2.24	11.23	2.19		
9	11.95	2.15	12.04	2.33		
10	10.81	2.18	11.87	2.30		
Generation means	11.60		11.79		11.65	

TABLE 13 (continued)

Generation 9

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.45	1.98	10.40	1.92	10.46	2.13
2	10.88	2.16	10.83	1.94		
3	10.66	2.12	9.47	2.04		
4	10.21	2.03	9.37	1.86		
5	10.27	1.94	9.75	1.84		
6	10.05	2.00	10.30	2.06		
7	10.59	2.23	9.85	2.15		
8	9.70	2.02	10.13	2.17		
9	9.95	1.74	10.32	2.02		
10	10.63	2.16	10.81	1.93		
Generation means	10.34		10.12		10.46	

Generation 10

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	9.07—	1.86	10.19	2.07	9.47	1.55
2	9.49	1.79	9.64	2.20		
3	9.27	1.98	10.03	2.14		
4	10.20	1.80	9.82	1.84		
5	9.13	1.69	10.00	1.60		
6	9.88	2.07	9.87	2.03		
7	9.54	1.68	9.98	1.93		
8	10.22	1.92	9.79	1.69		
9			9.84	1.99		
10	9.88	1.94	10.18	1.97		
Generation means	9.63		9.93		9.47	

TABLE 13 (continued)

Generation 11

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.82	2.09			12.60	1.93
2	12.30	2.25				
3	12.07	2.20				
4	11.32	2.14				
5	11.33	2.17				
6	12.23	2.19				
7	11.99	2.07				
8	11.75	2.10				
9	11.46	2.02				
10	10.95	1.83	11.87	2.13		
Generation means	11.62		11.87		12.60	

Generation 12

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.22	2.09	11.40	2.08	11.19	1.95
2	11.07	1.96	10.33	2.11		
3						
4	10.43	2.05	10.94	2.27		
5	10.76	2.12	10.24	1.78		
6	10.63	2.09	10.92	2.22		
7						
8	10.77	2.17	10.91	2.14		
9						
10	10.69	1.94	10.22	2.01		
Generation means	10.79		10.71		11.19	

TABLE 13 (continued)

Generation 13

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.53	2.04	9.77	1.76	10.37	2.05
2	10.07	1.87	10.49	2.16		
3	11.22	2.08	10.75	2.38		
4	10.61	2.33	10.65	1.69		
5	9.76	2.09	10.72	2.32		
6	10.52	2.20	10.04	1.99		
7	10.94	2.33	10.87	2.18		
8	10.66	1.93	10.94	2.19		
9	10.35	2.31	10.19	2.26		
10	10.83	2.25	9.63	2.32		
Generation means	10.55		10.41		10.37	

Generation 14

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.48	1.92	10.58	2.15	10.33	2.10
2	10.47	1.76	12.16	2.17		
3	10.64	1.82				
4	10.74	1.83	10.33	2.13		
5	11.98	2.55	14.85*	2.70		
6	12.52	2.33				
7	11.71	2.11	11.24	2.35		
8	12.29	2.16				
9	11.29	2.10	12.30	1.98		
10	11.68	2.22	11.76	2.22		
Generation means	11.48		11.90		10.33	

*Mutant.

TABLE 13 (continued)

Generation 15

CULTURES	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.67	2.11	11.78	2.08	11.80	2.46
2	11.78	2.07	11.47	1.76		
3	11.90	1.87	12.12	2.11		
4	11.34	2.34	11.65	1.91		
5	11.75	2.04	11.36	2.02		
6	11.33	2.33	11.07	1.79		
7	11.35	2.28	11.20	1.98		
8	12.50	2.24	11.71	2.06		
9	11.27	2.21	11.77	2.04		
10	11.22	1.85	10.47	2.19		
Generation means	11.61		11.46		11.80	

Generation 16

CULTURES	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.37	2.08	10.86	2.05	11.00	2.45
2	11.52	2.10	10.41	2.09		
3	10.50	2.07	10.79	2.21		
4	11.57	2.01	10.82	2.26		
5	11.27	2.07	10.55	1.97		
6	11.49	1.98				
7	11.17	2.33	11.25	1.96		
8	11.76	2.25	10.73	1.73		
9	12.09	1.78	10.22	1.65		
10	11.77	2.04				
Generation means	11.45		10.70		11.00	

TABLE 13 (continued)

Generation 17

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.70	1.98	9.97	1.63	10.34	1.81
2	9.83	1.82	10.60	1.93		
3	10.36	1.77	9.92	1.87		
4	10.46	2.01	10.09	1.75		
5	10.61	1.92	9.88	1.80		
6	10.32	2.16	10.37	1.87		
7	10.17	1.72	10.01	1.73		
8	10.87	1.67	10.17	2.00		
9	10.01	1.83	10.60	2.21		
10	9.80	1.96	10.29	1.86		
Generation means	10.31		10.19		10.34	

Generation 18

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	9.56	1.97			10.80	1.95
2	9.94	2.43	10.66	2.02		
3	10.47	1.82	9.92	1.54		
4	10.36	2.01				
5	10.00	1.78				
6	9.72	2.08	10.45	1.74		
7			10.46	1.93		
8						
9	10.02	2.16	10.26	1.74		
10	9.85	2.04	10.52	1.79		
Generation means	9.99		10.39		10.80	

TABLE 13 (continued)

Generation 19

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1			11.60	1.74	11.37	1.89
2	11.49	2.08	11.41	1.97		
3	11.12	2.02	11.22	1.97		
4	11.14	2.16	10.85	1.99		
5	11.12	2.18	10.72	1.69		
6	11.49	2.07	11.71	2.11		
7	11.69	1.96	11.91	2.28		
8	11.61	1.98	12.30	2.04		
9	11.44	1.99	12.15	1.98		
10			11.79	2.18		
Generation means	11.49		11.57		11.37	

Generation 20

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.59	2.05	11.35	1.91	10.79	1.81
2	10.89	2.05	11.11	1.86		
3	11.00	2.11	11.05	1.93		
4	10.55	1.93	11.14	1.94		
5	10.69	2.00	10.68	2.18		
6	11.22	1.90	11.41	1.96		
7	10.68	1.91	10.88	1.78		
8	10.98	2.14	10.95	1.81		
9	10.53	2.02	10.02	2.03		
10	10.86	2.07	10.49	2.10		
Generation means	10.90		10.90		10.79	

TABLE 13 (continued)

Generation 21

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.70	2.26	11.43	2.01	10.36	2.01
2	11.47	1.96	11.01	1.87		
3	10.49	1.92	9.64	1.70		
4	10.74	1.69				
5	10.80	2.09	10.86	2.00		
6			11.37	1.94		
7	10.78	1.98	10.88	2.10		
8	8.99	1.87	11.44	1.99		
9	11.95	2.09	10.46	2.02		
10	12.21	2.04	10.59	1.84		
Generation means	11.13		10.85		10.36	

Generation 22

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.76	2.07	11.96	2.00	10.58	1.94
2	11.22	2.10	10.85	1.99		
3	11.14	2.00	11.39	2.33		
4	11.06	1.74	10.75	2.26		
5	10.66	1.74	11.21	1.74		
6	11.11	1.95	11.76	2.32		
7	11.37	2.15	12.16	2.20		
8	11.42	2.14	11.56	1.67		
9	11.38	2.14	11.30	1.87		
10	11.04	1.83	11.80	2.30		
Generation means	11.22		11.47		10.58	

TABLE 13 (continued)

Generation 23

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.37	2.02	10.89	1.95	12.13	2.28
2	10.80	1.92	11.29	1.84		
3	11.29	1.77	10.96—	1.80		
4	11.50	1.80	10.82	1.81		
5	10.94	1.95	11.50	1.96		
6	11.30	1.83	11.53	1.77		
7			11.44	2.05		
8	10.98	1.90	11.28	1.86		
9	10.98	1.91	11.56	1.88		
10	10.87	1.82	11.46	1.99		
Generation means	11.11		11.27		12.13	

Generation 24

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.48	2.26	11.48	1.92	11.70	2.52
2	11.56	2.23	10.88	2.25		
3			10.40	1.89		
4			11.35	1.98		
5			10.63	2.02		
6			11.26	2.22		
7			11.11	2.00		
8			11.22	1.93		
9			11.37	2.14		
10			11.46	2.17		
Generation means	11.52		11.12		11.70	

TABLE 13 (continued)

Generation 25

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1			10.33	1.62	10.90	1.91
2			10.45	2.00		
3			11.45	2.23		
4			10.44	1.91		
5	10.58	2.17	10.46	1.96		
6			10.91	1.87		
7			10.72	1.85		
8	10.87	2.31				
9						
10						
Generation means	10.73		10.68		10.90	

Means of all cultures in experiment 2

PLUS SELECTIONS	MINUS SELECTIONS	INTERMEDIATE GROUP
11.330 ± 0.469	11.235 ± 0.043	11.39 ± 0.126
$\sigma = 1.06 \pm 0.033$	$\sigma = 0.97 \pm 0.030$	$\sigma = 0.034 \pm 0.089$

Difference between plus and minus selections $= 0.095 \pm 0.064$
Difference between plus selection and intermediate group $= 0.060 \pm 0.134$
Difference between minus selections and intermediate group $= 0.155 \pm 0.133$.

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GENETICS, MARCH 1922

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THE GALTON AND MENDEL CENTENARY

The year 1922 is notable as the centenary of the births of both FRANCIS GALTON and GREGOR MENDEL. An international celebration of the centenary of the birth of MENDEL is to be held in Brünn, Czechoslovakia, in September, and a portion of the scientific programs at the coming Convocation-Week meetings in Boston, U. S. A., will be arranged as a memorial to GALTON and MENDEL.

The coincidence of these two centenaries deserves to be signalized by the establishment of a permanent Memorial and the EDITORIAL BOARD OF GENETICS proposes for this purpose to raise by popular subscription a "GALTON AND MENDEL MEMORIAL FUND," the principal of which shall remain intact in perpetuity, the income only to be devoted to defraying the expense of occasional colored plates or other expensive types of engraving, as needed, and also to meeting a part of the heavy cost of publishing tabular matter so essential in a technical genetical journal.

In asking all subscribers to contribute liberally to this FUND, the two following considerations may be pointed out: (a) GENETICS is not published to make a profit for any one, and the proceeds of the MEMORIAL FUND will all go back to the subscribers in the enhanced value of the journal; (b) the only other technical genetical journal published in the English language charges $17.00 for 600 pages, while GENETICS charges only $6.00. Each subscriber to GENETICS can well afford to contribute some small multiple of the difference in subscription price between these two journals, since in this way can be best assured that the low subscription price of GENETICS will be continued, while its facilities for adequately meeting the publication needs of genetical investigators will be materially increased.

Subscribers are kindly requested to consider this a direct personal appeal to each, individually, to send as generous contribution as possible, without awaiting a special request by letter, thus saving a portion of the cost of direct correspondence.